Chapter 7
A Systems Biology Approach for Understanding Granuloma Formation and Function in Tuberculosis

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Abstract The pathologic hallmark of tuberculosis is the granuloma. A granuloma is a multifaceted cellular structure that serves to focus the host immune response, contain infection and pathology, and provide a niche for the bacillus to persist within the host. Granulomas form in response to *Mycobacterium tuberculosis* infection, and if a granuloma is capable of inhibiting or killing most of the *M. tuberculosis* present, humans develop a clinically latent infection. However, if a granuloma is impaired in function, infection progresses, granulomas enlarge, and bacteria seed new granulomas; this results in progressive pathology and disease, i.e., active tuberculosis. In clinical latency, immunologic perturbation at the level of the granuloma can result in reactivation of infection. In humans, there are a variety of granuloma types, even within the lungs of a single host.

The roles and interactions of various cells (macrophages, T cells, B cells, and neutrophils) and molecules (cytokines, chemokines, and effector molecules) within a granuloma are complex and challenging to address by experimental methods alone. Computational approaches, in particular agent-based modeling, can be used

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to dissect the temporal and spatial aspects of granuloma formation and function. Here we explain how a systems biology approach can integrate experimental and computational work to address critical questions necessary to understanding granulomas and contribute to the development and testing of strategies for prevention and treatment.

1 Introduction

1.1 Granuloma Formation and Function

Mycobacterium tuberculosis is primarily a respiratory pathogen, transmitted via aerosol from a person with active tuberculosis to another person. Once in the airways, the bacillus encounters various cells, primarily alveolar macrophages and dendritic cells (DCs). Although the early events in transmission are poorly understood, it is believed that many bacilli may be destroyed by alveolar macrophages or other airway cells. Surviving bacilli transit to the parenchyma, begin to replicate, and initiate an inflammatory response. Dendritic cells engulf bacilli and migrate to the thoracic lymph nodes (LNs) to prime a T cell response [1]. At least one thoracic LN becomes infected, and often stays infected (the classic Ghon complex is the initial lung lesion and the associated infected LN).

The inflammatory response in the lung likely results in recruitment of cells from the blood, including macrophages and neutrophils, which attempt to contain the infection, but also contributes to additional inflammation. In the LN, the T cell response takes at least 2 weeks to be primed [2–4], at which point the T cells migrate to the lungs and participate in granuloma formation. A granuloma forms in response to a chronic antigenic stimulus in the context of macrophage inflammation and is the pathologic hallmark of mycobacterial infections, including tuberculosis. A tuberculous granuloma can take many different forms (see Fig. 7.1), but is generally composed primarily of macrophages and lymphocytes, organized into a spherical structure (for review, see [5]). The classic caseous granuloma consists of a centrally necrotic area (grossly resembling cheese and hence the term "caseous"), surrounded by layers of macrophages, which are in turn surrounded by a smaller cuff of lymphocytes. The lymphocytic cuff can contain both CD4+ and CD8+ T cells, but B cells, including plasma cells, are also prominent. Multiple other cell types, including neutrophils, DCs, and fibroblasts, can also be found in granulomas.

There are several "types" of macrophages within granulomas, including epithelioid macrophages, foamy macrophages, Langhans' giant cells, classically activated macrophages, and alternatively activated macrophages (see [6] for review). The roles and functions of each macrophage subset in the granuloma in terms of control of bacterial proliferation and pathology are not known, but one can speculate that classically activated macrophages participate in bacterial killing while alternatively activated macrophages may be important in preventing excessive pathology or promoting fibrosis.

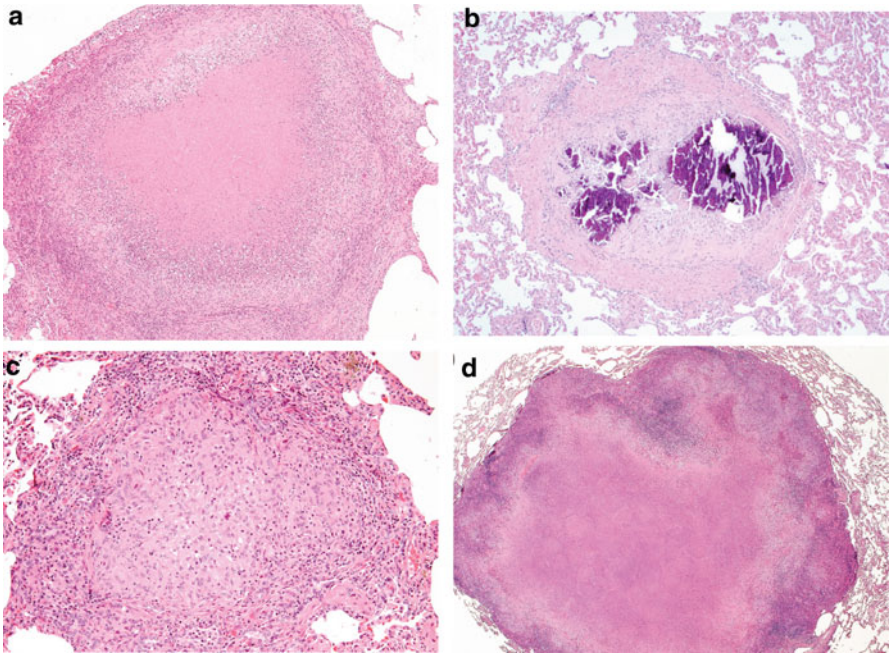

Fig. 7.1 Microscopic histopathology images of different types of tuberculosis granulomas from lungs of cynomolgus macaque model. (**a**) A caseous granuloma consisting of a central area of caseum surrounded by a mantle of epithelioid macrophages and peripherally located cuff of lymphocytes from a monkey with active disease, ×5 H&E. (**b**) A well-circumscribed non-necrotizing (solid cellular) granuloma consisting of a core of epithelioid macrophages and peripheral lymphocytes from a monkey with active disease ×5 H&E. (**c**) A fibrocalcific granuloma consisting of centrally located mineral (darkly staining) and fibroblasts ×5 H&E. (**d**) A large caseous granuloma from an anti-TNF antibody treated monkey at 8 weeks post-infection (×2, H&E). (**a**) and (**b**) were reprinted with permission from American Society for Microbiology and originally appeared in Lin et al., Infection and Immunity, 2009 Vol 77, p 4637 (Fig. 5) DOI 10.1128/iai.00592-09. (**d**) was reprinted with permission from John Wiley & Sons Publishing, and originally appeared in Lin et al., 2010, Arthritis and Rheumatism, Vol 62, p 344 (Fig. 3) DOI 10.1002/art.27271

In addition to the classic caseous granuloma, there are other types of granulomas seen in humans, including non-necrotic (composed primarily of macrophages with sparse lymphocytes), suppurative (neutrophil-rich), fibrotic (primarily fibroblasts), and mineralized (calcified often in the setting of caseous necrosis). All granuloma types, even mineralized granulomas, can be seen in active TB, although a person may have a predominance of one type or another. In latent TB, the granuloma types appear to be primarily caseous, mineralized, or fibrotic, rather than non-necrotizing [7]. In active TB, granulomas can be multifocal and coalescing, and large consolidations or TB pneumonia can also occur. Granulomas can also break through to an airway, resulting in cavity formation, which appears to be a major factor in transmission [8], as the cavity allows escape of large numbers of bacilli from granuloma

into the airways. Granulomas are also present in the thoracic LN that was initially infected, and more than one LN can be involved in a host with active or latent infection. Granulomas can also be seen in other organs, such as liver, spleen, brain, or bone in the case of extra-pulmonary disease.

The role of a granuloma from the host-centric point of view is to contain infection, destroy bacilli, and limit the pathology of the infection. A granuloma forms in response to infection, and granulomas are found in both active and latent *M. tuberculosis* infections, indicating that mere formation of a granuloma is insufficient to control the infection. The granuloma must function adequately to be a useful barrier to disease, i.e., to allow the host to either eliminate the bacilli completely or maintain sufficient control over bacterial replication that the infection remains clinically asymptomatic (i.e., latent infection).

From the mycobacteria-centric point of view, the granuloma serves a purpose as well. *M. tuberculosis* has evolved to persist within the granuloma for years, and can cause reactivation TB decades after the initial infection. Thus, the granuloma is a survival niche for the bacillus. In addition, the pathology caused by a granuloma, particularly one that results in cavity formation, is essential for efficient transmission of infection to a new host. It has been demonstrated in the zebrafish system that mycobacterial virulence factors actively participate in granuloma formation and cell recruitment [9], supporting the view that this structure is important in the pathogenesis of the infection.

Thus a granuloma, the hallmark of tuberculosis, is a structure that benefits both the host and the microbe: the central battle between host and microbe likely occurs at the level of the granuloma. Understanding and dissecting mechanisms, both host and bacterial, which occur during granuloma formation and function within each type of granuloma, will lead to a better understanding of this complex disease. This in turn will direct development of new therapeutic and preventive strategies to treat tuberculosis.

1.2 Key Cellular and Molecular Players Relevant to Granulomas

The immune responses induced by *M. tuberculosis* infection are myriad and complex, and even now it remains incompletely understood which responses are required for protection and which contribute to pathology [10, 11]. In truth, there is significant overlap among protective and pathologic responses, with the best outcome achieved by a balance of pro-inflammatory and anti-inflammatory responses, particularly at the level of the granuloma. It is well accepted that CD4+ and CD8+ T cells are important in defense against tuberculosis, although the mechanisms by which these cells provide protection are not completely understood. IFN-γ has been considered to be a key mechanism by which T cells activate macrophages for killing of bacilli [12, 13], although recent studies in mouse models suggest that CD4+ T cells can contribute in other ways as well [14, 15]. TNF is a cytokine that has been demonstrated to be

important in preventing progression of initial infection or reactivation of latent infection in several animal models, including non-human primates [16, 17]. The use of TNF inhibitors as treatment for inflammatory diseases in humans has confirmed that TNF is a major player in the protective immune response against tuberculosis [18]. However, TNF has numerous functions in the human immune response and sorting out which are the relevant mechanisms is difficult in vivo. Although the role of B cells and antibodies in tuberculosis has not been established, some studies support the view that these cells are important contributors to protection [19].

1.3 A Systems Biology Approach to Understanding Granuloma Formation and Function

Despite decades of research on TB, our understanding of the factors that lead to active, latent, and reactivation TB remains very much incomplete. A central hypothesis to our work is that these different infection outcomes are reflected locally at the level of the granuloma and that granuloma structure and function are the result of the interplay of events at organ, tissue, cellular, and molecular scales over the time course of minutes to years. For example, the structure of a granuloma in the lung is influenced by chemokines recruiting immune cells into the lung, by antigen presentation events occurring in the LN, by the circulation of dendritic and T cells between the LN and lung granuloma, by cell–cell interactions in the developing granuloma, and by the binding of TNF to receptors. In addition, mycobacterial virulence factors are known to actively participate in granuloma formation and cell recruitment [9], supporting the view that both bacteria and host factors are relevant to this process.

We describe in this chapter a systems biology approach to understanding granuloma formation and function. In this context, systems biology is a discipline at the intersection of immunology, microbiology, mathematics, engineering, and computer science that allows us to integrate experimental and computational approaches. The power of systems biology to address complex host and bacterial responses in infections such as tuberculosis is vital to enhancing our understanding of these diseases and identifying factors to target for prevention or treatment. In the particular case of granuloma formation and function during *M. tuberculosis* infection, systems biology may help us in many ways to identify the mechanisms involved in *M. tuberculosis*–host dynamics. For example, models in which TNF–TNF receptor interactions are represented can help determine their role in influencing containment of bacteria by a granuloma, or, through the use of analysis tools, can identify potential immunological targets for immunotherapy. We describe below both animal and computational models that are being used to study granuloma formation, and then turn to the key insights these models provide.

2 Experimental and Computational Models of Tuberculosis Granulomas

2.1 Experimental Models

The TB field is extremely fortunate to have several different and well-characterized animal models for study of this disease (reviewed in [20]). The most commonly used model is the mouse. The availability of reagents and especially genetically modified animals (lacking different molecules, cytokines, or cell types) has provided invaluable information about virulence factors of the pathogen and host responses to the infection. Other advantages of mice include the relatively inexpensive cost of housing them in a Biosafety Level 3 facility, the lack of coughing (and therefore transmission), the inbred strains that limit variability among animals, and the ease of infecting, manipulating, and handling them. However, there are distinct limitations to the mouse models of TB. Mice become chronically and progressively infected with *M. tuberculosis*, and every infected mouse eventually succumbs to the disease. Thus, unlike in humans where latent infection is observed in the majority of cases, there is no true latent infection in mice. Although there are several "latency" models put forth over the years [21], these are all dependent on some type of manipulation (e.g., anti-TB drugs), and there is little indication that they resemble human latent infection. In addition, the mouse granuloma, which is best characterized as granulomatous inflammation, is substantially different from the human granuloma in terms of structure and organization, the lack of caseous necrosis, and an absence of cavity formation. Thus, studying mouse granulomas as a model for human granulomas can be problematic, since many of the features and microenvironments of human granulomas are absent in mice. There have been a few newer mouse models using genetically manipulated mice that recapitulate certain features of human granulomas and these hold some promise for granuloma studies [22]. Another rodent, the rat, has also been described as a model of TB [23].

Guinea pigs have also long been used as a model of tuberculosis, especially for vaccine studies [24, 25]. These animals are very susceptible to *M. tuberculosis* infection, and all proceed to death from tuberculosis after months of infection. The granulomas in Guinea pigs include inflammation similar to mice, but also more structured caseous and mineralized granulomas. There are several elegant studies on the pathologic features of Guinea pig granulomas [26–28]; however, immunologic manipulation of Guinea pigs is still very challenging, and the tools for studying host responses, although improving, remain limited.

Rabbits are also used in TB research, both historically and currently [29–32]. There are several interesting features of rabbits, including their relative resistance to many laboratory strains of *M. tuberculosis*, their exquisite susceptibility to *M. bovis*, the presence of caseous lesions that form and resolve in a relatively homogeneous manner, and the propensity for cavity formation. The lack of immune reagents limits this model, as does the size and containment needs of rabbits.

Zebrafish (and other fish) were developed as a model for tuberculosis over the past 15 years, and have many attractive features [33]. Zebrafish embryos are

transparent, which allows one to visualize granuloma formation in various parts of the fish body. The fish form caseous granulomas, primarily composed of macrophages. The ability to manipulate the fish genetically and to examine the contributions of genes in high-throughput, forward genetic screens has provided valuable insights into the host–pathogen interaction of mycobacterial infections. However, the natural species that infects fish is *M. marinum*, not *M. tuberculosis*. Although there are substantial similarities between the two species, there are also differences that may contribute to difficulty in translating the findings in *M. marinum* models to human tuberculosis.

Non-human primates have been used for decades to address key aspects of tuberculosis. Many years ago, macaques were used in drug studies of tuberculosis. After a hiatus of a few decades, this model has reemerged as an important contributor to translational studies of tuberculosis, including drug, vaccine, pathogenesis, and immunologic studies. Low dose *M. tuberculosis* infection of cynomolgus macaques results in the full spectrum of human *M. tuberculosis* infection outcomes, from latent to active TB [7, 34]. In addition, reactivation of latent *M. tuberculosis* infection has been demonstrated after TNF neutralization [16], CD4+ T cell depletion [35], and SIV coinfection (as a model of TB and AIDS) [36, 37]. More recently, a model of latent infection in rhesus macaques has been reported, using a low-virulence strain of *M. tuberculosis* [38]. The full spectrum of human pathology is also observed in the macaque, with all varieties of granulomas observed, in both lungs and LNs, and cavity formation [5, 7]. These two features (spectrum of disease outcomes and pathology identical to humans) make this an important and useful model for studying human tuberculosis. Another feature of macaques that adds to their value as a model is the wide availability of immunologic reagents, for assessing immune responses (peripherally and in organs) and for manipulating the system. However, there are several limitations to this model system. First, there is extensive genetic variability among monkeys, requiring larger cohorts of animals to obtain statistically significant results. Second, housing macaques under Biosafety Level 3 conditions is a challenge, as these animals can cough and transmit infection to other animals and humans, and primate BSL3 facilities are not available at most institutions. Third, the cost of purchase and husbandry makes many experiments prohibitively expensive. Every effort must be made to obtain as much data as possible from each animal, and sharing of tissues and samples among other investigators allows more labs to take advantage of this resource. The cost also makes it difficult to obtain tissue samples at all the desired time points; however serial peripheral samples can be obtained from the same animal, which is ideal for matching to human studies.

A chief advantage of animal models is the ability to obtain samples from the site of disease, i.e., the lung and thoracic LNs, including granulomas. This is extremely challenging in human studies, and a model that is similar to humans provides an opportunity to assess events at the granuloma level. The types of samples and data that can be obtained include cells (numbers, phenotypes, and functions), cytokines (levels and sources), and spatial location of cell types and cytokines (using, for example, immunohistochemistry). Bronchoalveolar lavage, which allows one to sample airways serially, and blood can be obtained easily and frequently from the larger animals, as well as peripheral LNs.

More recently, imaging of live animals has been used to serially assess events during infection. Fluorescent imaging (fish and other animals), video monitoring (zebrafish), and PET/CT imaging (mice, rabbits, and monkeys) have been applied to tuberculosis models [35, 39] (Via and Barry, unpublished) and provide the unique opportunity to "watch" the infection evolve over time at the level of the granuloma, and in some cases to determine where the bacilli are during infection. These new imaging tools open up new possibilities for understanding *M. tuberculosis* infection, and in some cases can also be performed in humans (e.g., PET/CT). All of these methods can provide quantitative, serial, and spatial data for incorporation into computational models.

In addition to animal infection models, the use of in vitro granuloma models [40] and in vivo models using non-replicating agents [41, 42] provides unique insight into processes involved in granuloma formation and function. For example, mycobacterial antigen-coated beads are used to induce pulmonary granulomas with cytokine and cellular patterns that closely match those in an active mycobacterial infection [43, 44].

2.2 Computational Models of Tuberculosis Granuloma

Mathematical and computational modeling provides a unique approach to studying the behavior of complex biological systems. These methods can be used to better explore hypothesized mechanisms, generate and test new hypotheses, run virtual (in silico) experiments, interpret data, motivate particular experiments, and suggest new drug targets. A series of mathematical and computational models have been developed to investigate the host response to *M. tuberculosis* infection [45–57]. In particular, model-based analysis of the formation and function of a TB granuloma contributes to understanding the mechanisms that control the immune response to *M. tuberculosis* [45–49, 51, 57]. These models complement experimental approaches and can be used to address questions in TB that are difficult or currently impossible to approach experimentally. The high cost and time investment needed to fully explore many interacting immune factors and various outcomes involved within the *M. tuberculosis*–host interactions in an experimental setting are factors that alone should promote the use of computational models. Building computational models can also allow us to integrate data derived from experiments on different tissues, different biological scales (e.g., molecular or cellular), and different timescales into a comprehensive picture of the immune response to *M. tuberculosis*.

Differential equation (DE)-based models typically describe a deterministic relationship among several continuously varying quantities (e.g., numbers of cells and concentrations of molecules) and their rates of change in space and/or time. We have developed DE-based models for studying temporal dynamics of cytokines and effector cells during the immune response to *M. tuberculosis* [52, 53, 58, 59]. These models are based on known interactions of immune cells in the lung during *M. tuberculosis* infection. Experimental data are used to estimate parameter values.

When data are not available, uncertainty and sensitivity analyses are used to define parameter spaces. Uncertainty analysis is performed to investigate the uncertainty in the model output that results from uncertainty in input parameter values. Sensitivity analysis is then used to quantify how input uncertainty (e.g., biological variability coupled to unknown ranges of variation for model parameters) affects model outcomes and to identify critical model parameters. Once validated against experimental data, the models are used to make novel predictions about dynamics, progression of infection, and potential therapies. Examples of contributions these models have made to our understanding of TB include identifying the critical impact of delays in either DC migration to the draining LN or T cell trafficking to the site of infection on the outcome of infection [58], and identifying the key role of cytokine IL-10 in balancing macrophage phenotypes in the lung and LN [59]. DE models can also be used to examine spatial aspects of the immune response, including analysis of the process of granuloma formation and cytokine availability in a granuloma [42, 51].

In contrast to DE-based models, agent-based models (ABMs, also known as individual based models) are rule-based models that capture a variety of stochastic and discrete events occurring in the immune system. An ABM has the following components: *agents* (e.g., immune cells and bacteria), the *environment* where agents reside (e.g., a two-dimensional grid representing a section of lung tissue), the *rules* that govern the dynamics of agents, including movements, actions, and interactions between agents as well as between agents and environment, and *timescales* on which the rules are executed. In an ABM, the local, possibly stochastic interactions occurring at the level of agents lead to global, system-wide dynamics and emergent spatial and temporal patterns. Hence, ABMs are particularly useful for studying complex systems such as TB granulomas in which cell heterogeneity and spatial interactions are important.

We developed first- and second-generation ABMs to describe the immune response to *M. tuberculosis* and to identify mechanisms that control granuloma formation and function [46, 47]. Next-generation granuloma ABMs were developed in response to new biological data that indicated the importance of including additional cell types (e.g., effector CD8+ T cells and regulatory T cells), cytokines (e.g., TNF and IL-10), and chemokines (e.g., CCL2, CCL5, and CXCL9) [45, 46, 57]. The major cell types, biological activities, and interactions captured in our current granuloma ABM are listed in Table 7.1. An overview of selected ABM rules governing cellular activities and interactions on a grid representing a section of lung tissue is presented in Fig. 7.2. These models are the first to track the dynamics of formation and maintenance of a granuloma in space and time, simultaneously providing critical details regarding cellular interactions and molecular concentrations. There are no experimental methods to obtain these detailed, continuous data in primates.

A critical aspect of studying mechanisms underlying the formation and function of a granuloma during *M. tuberculosis* infection is the integration of information across multiple biological scales (molecular, cellular, tissue/organ, and host scales; see Fig. 7.2). Immunity to *M. tuberculosis* in humans and animal models has been attributed to activities of a variety of cytokines, including TNF, IFN-γ, and IL-10 (reviewed in [60]).

Table 7.1 List of agents, biological activities and interactions captured in the lung granuloma agent-based model and lymph node DE-model

Cell types and states	Activities and interactions in the lung granuloma (ABM)	Activities and interactions in the lymph node (DE model)
Macrophage	Death due to age Chemokine-dependent movement	N/A
Resting macrophage (M_r)	TNF and chemokine-dependent recruitment STAT-1 activation due to interaction with T_γ Uptake of extracellular *M. tuberculosis* (either kills *M. tuberculosis* or gets infected ($M_r \rightarrow M_i$)) Macrophage activation ($M_r \rightarrow M_a$) if both STAT-1 and NF-κB are activated and M_r is not down-regulated by T_{reg}	N/A
Infected macrophage (M_i)	Growth of intracellular *M. tuberculosis* Uptakes extracellular *M. tuberculosis* Gets chronically infected ($M_i \rightarrow M_{ci}$) with growth of intracellular *M. tuberculosis* STAT-1 activation due to interaction with T_γ Macrophage activation ($M_i \rightarrow M_a$) if both STAT-1 and NF-κB are activated and M_i is not down-regulated by T_{reg}	N/A
Chronically infected macrophage (M_{ci})	Growth of intracellular *M. tuberculosis* Bursts and disperse *M. tuberculosis* to the environment due to intracellular growth of *M. tuberculosis*	N/A
Activated macrophage (M_a)	Kills extracellular *M. tuberculosis* effectively	N/A
T cell	Death due to age Chemokine-dependent movement TNF and chemokine-dependent recruitment	Death due to age Recruitment, re-circulation and migration Differentiation
Naïve CD4+ and CD8+ T cells	None	Natural turnover and enhanced recruitment induced by DC arrival Differentiation to precursor effector cells (Th0 and T80) after interacting with DCs

Precursor effector T cells	None	Proliferation and migration to the blood Further differentiation to fully effector CD4+ and CD8+ T cells
Pro-inflammatory T cell (T_γ)	If not down-regulated, can activate STAT-1 in M_r and M_i If not down-regulated by a T_{reg}, can mediate Fas/FasL-induced apoptosis of M_i and M_{ci}	Migrate out of the LN into the blood Represented in the LN by the sum of Th1 (i.e. fully effector CD4+ T cells) and T8 (i.e. fully differentiated IFN-γ producing CD8+ T cells)
Cytotoxic T cell (T_c)	If not down-regulated by a T_{reg}, can kill M_i and M_{ci} (perforin/granulysin-mediated mechanism)	Migrate out of the LN into the blood Represented in the LN by the fully differentiated cytotoxic CD8+ T cells, labeled as CTL
Regulatory T cell (T_{reg})	Down-regulate the actions of T cells and macrophages	Migrate out of the LN into the blood Represented in the model as 10% of the sum of Th1 and T8
Bacteria (*M. tuberculosis*)	Grow inside M_i and M_{ci} and in extracellular spaces Extracellular *M. tuberculosis* can infect M_r and M_i Extracellular *M. tuberculosis* can induce NF-κB activation in M_r and M_i	None
Molecules		
TNF	Secreted from M_i, M_{ci}, M_a, NF-κB-activated M_r, T_γ and T_c with different rates Degrades and diffuses in the environment (among grid micro-compartments) Induces NF-κB activation in M_r and M_i Induces apoptosis in all cells	None
Chemokines (CCL2, CCL5, CXCL9/10/11)	Secreted from M_i, M_{ci}, M_a, and NF-κB-activated M_r with different rates Degrades and diffuses in the environment (among grid micro-compartments)	None

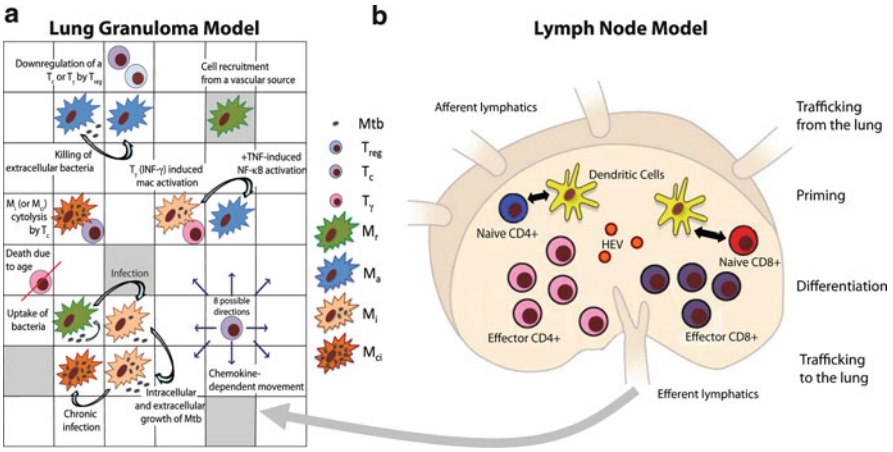

Fig. 7.2 Computational modeling of immunological processes within the lung and lymph nodes during *M. tuberculosis* infection. (**a**) An overview of selected ABM rules governing biological activities and interactions among immune cells and *M. tuberculosis* on a grid representing a section of lung tissue. Cell types and status are shown, as indicated on the *right* side of panel (**a**): M_r resting macrophage, M_i infected macrophage, M_{ci} chronically infected macrophage, M_a activated macrophage, *M. tuberculosis*: mycobacteria, T_γ pro-inflammatory IFN-γ producing T cell, T_c cytotoxic T cell, T_{reg} regulatory T cell. A complete description of all ABM rules is provided in [45]. (**b**) An overview of main mechanisms captured in the ODE-LN model. Dendritic cells migrate from the lung to the LN upon bacterial uptake and maturation. They traffic into lymphatic vessels and enter the T cell zone of the LN through afferent lymphatics. Once in the T cell zone of the LN, they interact with naïve CD4+ and CD8+ T cells [continuously circulating in the LN through high endothelial venules (HEV)], and eventually prime and activate them. Upon activation, T cells start to proliferate and differentiate into effector lymphocytes. These effector immune cells then migrate back to the lung, exiting the LN through efferent lymphatics. A complete description of all ODE-LN model rules is provided in [57]

These molecules are secreted from cellular sources in response to pathogen and host signals, interact with receptors on target cells, and trigger intracellular signaling pathways controlling cellular activities that ultimately contribute to formation of granulomas and immunologic control of *M. tuberculosis* infection. For example, TNF is secreted from infected and activated macrophages, interacts with TNF receptors (TNFRs) on the membrane of macrophages, and induces the NF-κB signaling pathway, leading to secretion of chemokines, a key process that attracts immune cells to the site of infection and influences their movement within a granuloma.

Agent-based modeling can provide a framework for describing these events. We capture cellular and tissue scale dynamics (see Fig. 7.2a) via well-described and probabilistic rules for interactions between immune cells and *M. tuberculosis*. Single-cell molecular scale processes (e.g., those controlling TNF/TNFR binding and trafficking) for each individual cell are captured by a set of ordinary DEs. Using this approach, we are able to, for example, alter a molecular property (e.g., TNF/TNFR binding affinity) and study its impact on a tissue scale outcome (e.g., size of the granuloma). As an example, we review below our recent studies on the multi-scale analysis of the role of TNF activities in controlling granuloma formation and function.

As a complex multi-scale process, granuloma models include parameters describing a large number of biological events. Hence, it is critical to understand the role that each of these parameters plays in determining how a granuloma functions. We have developed a number of useful and powerful tools to analyze these complex model systems. One approach is to perform *virtual* deletion and depletion experiments that mimic experimental gene knockout or molecule depletion studies. Loss of activity is achieved by setting relevant parameters (e.g., probabilities or rate constants) to zero or raising relevant thresholds to an unattainable level. Virtual deletion refers to the loss of activity from the beginning of simulation and virtual depletion refers to the loss of activity after establishment of a granuloma. A second approach is to use uncertainty and sensitivity analysis, which we have adapted for use in agent-based models [61]. We use uncertainty and sensitivity analysis in computational models of *M. tuberculosis* infection to analyze the impact of parameters describing events at different scales (molecular, cellular, tissue, or organ scales) on model outputs describing granuloma outcomes.

3 What Are Examples of Questions That Systems Biology Can Address?

3.1 Which Factors Influence the Ability of a Granuloma to Control Infection?

As described earlier (see Fig. 7.1), there are different types of granulomas in the lungs of non-human primates and humans with active TB [7]. Our granuloma ABM is able to recapitulate granulomas with different abilities to control infection by varying values of important model parameters. Examples of these outcomes (as shown in Fig. 7.3) include containment (control of infection within a well-circumscribed granuloma containing stable and low bacterial levels), clearance, and uncontrolled growth of bacteria.

Many immune factors are critical to a protective immune response to *M. tuberculosis* infection in animal models (reviewed in [60]). These factors include priming and activation of antigen-specific T cells [3], production of chemokines contributing to recruitment of immune cells to the site of infection [62–64], and production of cytokines such as IFN-γ and TNF [10, 17, 60, 65]. Simulations of TNF, TNF receptor 1 (TNFR1), and IFN-γ gene knockouts and deletion/depletion of T cells (described in detail in [45, 46]) lead to uncontrolled growth of *M. tuberculosis* and formation of granulomas with irregular structures that include very high numbers of extracellular bacteria, large numbers of infected macrophages, and widespread caseation. These simulations indicate that the model captures important aspects of the biology of the immune response to *M. tuberculosis*.

In addition to validating the model with experimental data, we use simulations to perform novel virtual experiments. Such studies can predict critical components of

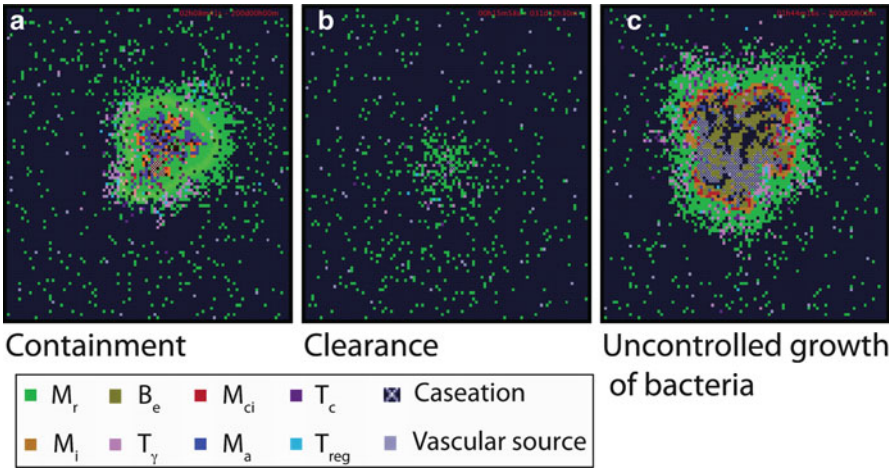

Fig. 7.3 Reproduction of possible outcomes of *M. tuberculosis* infection by the granuloma ABM by varying important model parameters. Granuloma snapshots for (**a**) a scenario of containment at 200 days post-infection, (**b**) clearance of *M. tuberculosis* in approximately 5 weeks as a result of an efficient immune response, and (**c**) a scenario of uncontrolled growth of bacteria as a result of knocking TNF out at 200 days post-infection. Cell types and status are shown by different *color squares*, as indicated at the *bottom* of the figure (M_r resting macrophage, M_i infected macrophage, M_{ci} chronically infected macrophage, M_a activated macrophage, B_e extracellular bacteria, T_γ pro-inflammatory IFN-γ producing T cell, T_c cytotoxic T cell, T_{reg} regulatory T cell). Caseation and vascular sources are also indicated

an effective immune response and can ultimately guide the design of laboratory experiments. For example, sensitivity analysis helps us predict the relative importance of various immunological processes (e.g., recruitment and movement of T cells, secretion, diffusion, and degradation of chemokines, and macrophage–T cell interactions) in immunity to *M. tuberculosis* and may suggest novel targets for control and therapy of TB. Our granuloma ABM identified cellular and tissue scale processes that significantly control bacterial numbers, caseation, and size of a granuloma: *M. tuberculosis* growth rates, activation of macrophages by IFN-γ producing T cells, and T cell movement and recruitment in lung tissue [45, 46]. Further, our model predicts molecular scale processes that have significant impact on granuloma outcomes. These processes include events at the level of TNF signaling and trafficking. Thus, we focus in the next section on our model-based findings regarding the role of TNF in granuloma formation and function.

3.2 What Is the Role of TNF in Granuloma Formation and Function?

The pleiotropic cytokine TNF functions as a critical part of the immune response to *M. tuberculosis* infection (see Sect. 7.1). Initial data identifying the roles of TNF

include TNF knockout/neutralization and TNFR1 knockout experiments in mice [17, 62, 66]. Based on these mouse studies, TNF was long believed to be essential for formation of granulomas. However, recent studies in zebrafish and non-human primate models of TB have shown that TNF, although not required for the formation of granulomas, is necessary to restrict bacterial growth in a granuloma [16, 67]. This suggests that TNF activities *within* a granuloma determine a granuloma's ability to immunologically restrain bacteria. This is confirmed by studies in humans, where TNF neutralization leads to reactivation of latent TB; pathologic studies in a subset of humans support the view that granuloma formation is maintained in the absence of TNF, but disease exacerbation and dissemination occur, indicating a failure of the granuloma to control bacterial replication [68]. Which mechanisms control TNF availability and activities in a granuloma, and how do these activities affect granuloma function during the long-term immune response to *M. tuberculosis*? Here, we present predictions of our approach resulting from integrating experimental (animal model) data and theoretical tools to address these questions.

3.2.1 Prediction I: Establishment of a TNF Concentration Gradient Within a Granuloma

Availability of TNF within a TB granuloma has been proposed to have a key role in the protective immunity to *M. tuberculosis*, although measuring the true TNF production and consumption within a granuloma is not yet feasible. The total TNF concentration at any one time in a granuloma has been estimated by cytokine bead array technology [7], although this is simply a snapshot of the concentration at necropsy of the animal. We calculate the TNF concentration in a granuloma using two different models: a DE-based model that considers a simple representation of the spatial structure of a granuloma at steady state and the granuloma ABM described earlier. Both models explicitly include single-cell level TNF/TNFR binding and trafficking processes (i.e., synthesis, internalization, recycling, and degradation of ligands and receptors), as these processes are critical to determining TNF concentration.

Values of some model parameters, including TNF/TNFR kinetic rate constants, are estimated from the literature. Other model parameters were determined based on a simple experimental system for granuloma formation in mice. The formation of granulomas was induced in mice following injection of Sepharose beads covalently coupled to *Mycobacterium* purified protein derivative antigen [43, 69]. The cellular composition of granulomas, TNF secretion rate, and TNFR densities on different types of cells were measured for these mouse granulomas. Our experiments indicate that macrophages and DCs are the major TNF-producing immune cells within a granuloma. Further, DCs, macrophages, and B cells are found to be the major TNFR-expressing cells.

Our granuloma ABM simulations based on these data suggest that there is a TNF concentration gradient in granulomas, such that the highest concentration occurs at the center of a granuloma [42] (Fig. 7.4). This gradient results from the emergence

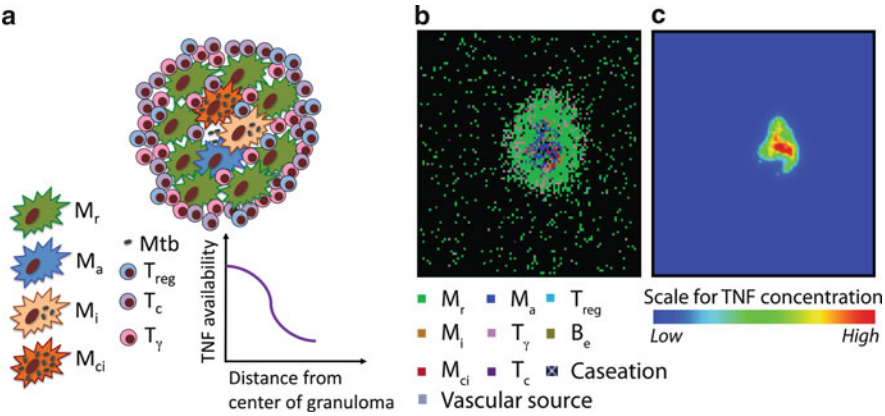

Fig. 7.4 Prediction of a TNF concentration gradient within a granuloma. This gradient, as verified by both the differential equation model [42] (**a**) and the agent-based model [45] (**b**), results from a granuloma with a specific cellular organization composed of a core of infected, activated, and resting macrophages surrounded by a ring of lymphocytes as well as TNF/TNFR binding and intracellular trafficking processes. Cell type abbreviations are as described in Fig. 7.3

of a specific organization of immune cells within a granuloma (i.e., concentration of infected macrophages at the core and concentration of lymphocytes at the periphery of the granuloma; see also Fig. 7.1) and the processes of TNF/TNFR binding and intracellular trafficking. What might the impact of this gradient be? The gradient could allow the spatial coordination of TNF-induced biological activities (i.e., activation of NF-κB and apoptotic signaling pathways) within a granuloma. Higher concentrations of TNF in the center of granuloma could induce caspase-mediated apoptotic pathway that favors antigen cross-presentation as well as the elimination of pathogen inside infected macrophages. However, very low levels of TNF at the periphery of the granuloma, although unable to induce apoptosis, are sufficient to turn on the NF-κB signaling pathway that favors cell survival and expression of pro-inflammatory genes in T cells.

3.2.2 Prediction II: A Critical Role for TNFR1 Internalization Kinetics

Experimental data suggest that TNFR1 internalization plays a key role in regulation of TNF signaling and mediates the process of TNF-induced apoptosis [70, 71]. Our simulations also predict a key role for TNFR1 internalization in control of the local TNF concentration and regulation of TNF activities during granuloma development [42, 45]. Further, the rate of TNF-induced internalization of TNFR1 regulates cell infiltration by affecting the extent and dynamics of TNF-dependent recruitment and activation of immune cells [45]. These are essential factors that control the level of inflammation in tissue. The importance of these factors to infection outcome at the level of a granuloma is demonstrated with our granuloma ABM: taken together, snapshots of model simulations (Fig. 7.5) and sensitivity

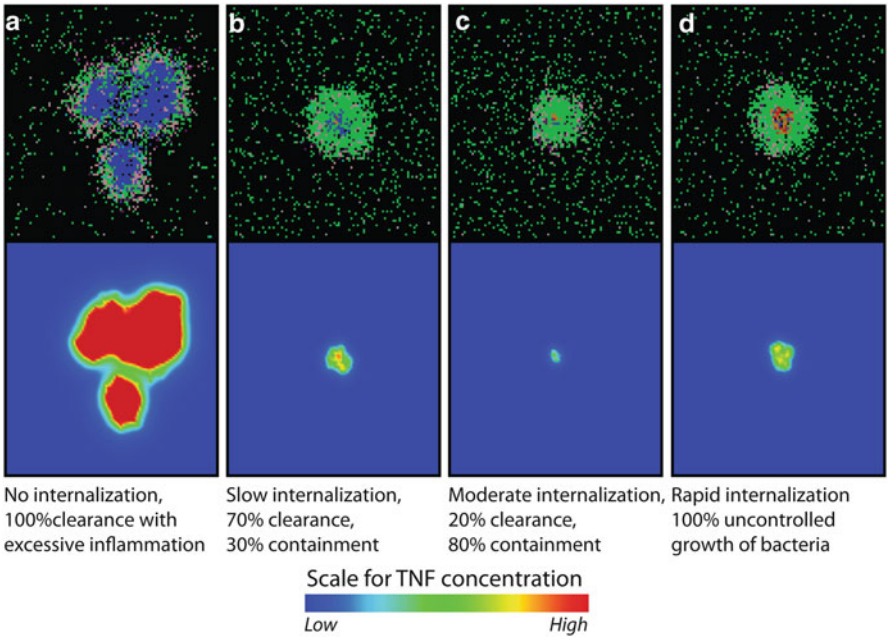

Fig. 7.5 Prediction of a key role for TNFR1 internalization kinetics in control of bacterial load and inflammation during *M. tuberculosis* infection. (**a**)–(**d**) Granuloma outcomes and tissue levels of TNF early after recruitment of T cells for varying rates of TNF-induced TNFR1 internalization. The *colors* representing cells of different type and status in granuloma snapshots are the same as those shown and defined in Fig. 7.3

analysis results demonstrate that TNF-induced TNFR1 internalization plays an important role in preventing excessive inflammation in tissue. This effect is particularly highlighted in Fig. 7.5a in which removal of the process of TNFR1 internalization leads to uncontrollably high tissue concentrations of TNF and very high rates of macrophage activation. Increasing the rate of TNFR1 internalization, however, controls the level of macrophage activation and tissue concentration of TNF (Fig. 7.5b–d).

TNFR1 internalization kinetics are also predicted to have a significant impact on bacterial numbers within a granuloma [45]. As highlighted in Fig. 7.5b–d, increasing the rate of receptor internalization reduces the rate of bacterial clearance. This effect results from reduced rates of TNF-induced activation of macrophages, diminishing their ability to kill bacteria. Overall, our results suggest the novel hypothesis that TNFR1 internalization kinetics play a role in balancing inflammation and bacterial killing within a granuloma, controlling whether there is clearance of bacteria, excessive inflammation, containment of bacteria within a stable granuloma, or uncontrolled growth of bacteria. This hypothesis can be tested in future studies investigating approaches to control and therapy of TB, as a number of ways have already been proposed to control the rate of TNFR1 internalization in vitro [70, 72, 73]

3.2.3 Prediction III: A Critical Synergy Between Individual TNF Activities

TNF has been experimentally characterized to have the following activities in *M. tuberculosis* infection: (1) macrophage activation (essential for killing of bacteria) [74, 75], (2) induction of apoptosis [76, 77], (3) induction of chemokine and cytokine production [63], and (4) regulation of cellular recruitment via transendothelial migration [78]. The use of computational modeling to describe how TNF regulates the process of granuloma formation provides an opportunity to investigate the importance of each of the TNF activities, separately or in combination, during the long-term immune response to *M. tuberculosis*. We can simulate in silico any combination of gene knockouts or deletions of biological activities by setting values of relevant parameters to either zero or very large values (infinity) in our model [46]. In particular, we simulate all 15 possible combinations in which at least one of the four TNF activities is deleted. For each case, we report the total number of bacteria and the maximal fraction of macrophages present that are activated in the granuloma (Fig. 7.6). Macrophage activation is considered here as a metric to assess the level of inflammation in tissue.

Our results demonstrate a synergy between TNF activities that contribute to control of infection within a granuloma [46]. As highlighted in Fig. 7.6, deletion of TNF-dependent activation (act^-), secretion ($secr^-$), or recruitment ($recr^-$) activities significantly increases bacterial levels within the granuloma. Among these activities, simulation of act^- leads to significantly higher bacterial numbers. This highlights the relative importance of TNF-induced macrophage activation in control of infection compared to other TNF activities. Double and triple deletions of these activities further exacerbate infection compared to single deletion simulations. The highest level of bacteria is observed when TNF-induced activation, TNF-induced secretion of chemokines, and TNF-induced apoptosis of immune cells are all simultaneously deleted ($act^-\ secr^-\ apopt^-$). Interestingly, deletion of TNF-induced apoptosis activity alone ($apopt^-$) does reduce bacterial numbers. This occurs as a result of high levels of macrophage activation in lung tissue that is accompanied by high levels of TNF concentration and cell recruitment. Thus, TNF-induced apoptosis reduces inflammation by controlling the level of macrophage activation at the expense of impairing bacterial clearance. It is this type of nonintuitive result that cannot be predicted without the use of computational models in tandem with experimental models.

3.3 What Are the Mechanisms Underlying TB Reactivation Following Anti-TNF Therapies?

M. tuberculosis can persist for decades within the lungs of humans. This results from a latent state of infection that represents a dynamic equilibrium between host and bacteria [79]. Disturbance of this equilibrium may lead to a failure of a granuloma to contain bacteria and progression to active TB, termed reactivation.

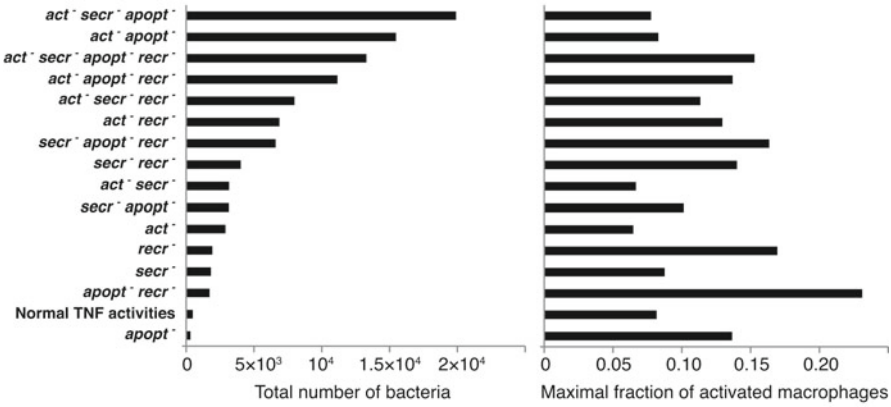

Fig. 7.6 Prediction of a synergy between four TNF-mediated biological activities: macrophage activation (*act*), inducing apoptosis (*apopt*), inducing recruitment of immune cells (*recr*), and inducing secretion of chemokines and cytokines (*secr*). Model predictions for total number of bacteria (*left*) and maximal fraction of macrophages that become activated (*right*) during granuloma development after *M. tuberculosis* infection are displayed for 16 possible scenarios with (+) or without (−) each of the TNF activities

For example, because of the inflammatory nature of TNF, treatment with TNF inhibitors (TNF-neutralizing drugs) is used in patients with inflammatory diseases such as rheumatoid arthritis and psoriasis. However, anti-TNF treatment has been recognized as one of the risk factors for reactivation of latent TB in humans. An increased incidence of TB has been reported among patients receiving treatment with TNF-neutralizing drugs [18, 80–82]. These drugs are either anti-TNF monoclonal antibodies such as infliximab, adalimumab, and certolizumab or soluble TNF receptor fusion proteins such as etanercept [83]. Although these drugs are similarly effective in treatment of some (but not all) inflammatory diseases [84, 85], the risk of TB reactivation posed by antibody-type drugs is several-fold greater than that for the soluble TNF receptor-type drugs [86–89]. A systematic and comprehensive comparison of TNF-neutralizing drugs with the aim of elucidating drug-specific reactivation mechanisms (especially in humans) has not been performed to date. Experiments required for a comprehensive analysis of the effects of drug characteristics (including TNF binding kinetics and stoichiometry, together with blood concentration and drug permeability into lung tissue, and apoptotic activities of antibody-type drugs) on the immune response to *M. tuberculosis* are at present impossible in vivo.

The granuloma ABM we described earlier, with modifications, can be used to investigate mechanisms by which TNF-neutralizing drugs interfere with granuloma function and thus immunity to *M. tuberculosis* [90]. In an earlier work, we used a DE-based model and found that the bioavailability of TNF is central to control of infection [52]; to address the mechanisms that control bioavailability during anti-TNF treatment, the ABM framework is useful. TNF-neutralizing drugs and their relevant properties can be incorporated into the ABM in a manner similar to TNF

itself. The dosing of a host with drug and the ability of that drug to cross from the bloodstream into the lung (permeability) and ultimately into the granuloma are also featured in this next-generation granuloma ABM. Our computational model thus links the dynamics of molecular scale drug/TNF/TNFR interactions to cellular and tissue/scale events occurring during granuloma formation and maintenance in the lung. Using this model, we identify functional and biochemical characteristics underlying the higher likelihood of TB reactivation that occurs for some TNF-neutralizing drugs. These characteristics include TNF *binding properties* (including affinity, binding/unbinding kinetics, stoichiometry, and ability to bind membrane-bound TNF (mTNF)), *permeability* (from blood vessels into lung tissue and penetration into the granuloma), and *apoptotic and cytolytic activities* that are reported for antibody-type drugs.

Our model-based analyses lead to novel and interesting hypotheses regarding drug-induced TB reactivation at the granuloma scale (Fig. 7.7). First, we find that the ability of a drug to bind mTNF is a major factor impairing granuloma function, leading to TB reactivation. This is because the cell membrane provides a scaffold on which TNF is available at a high concentration for neutralization before it is released as soluble TNF (sTNF) and diluted in extracellular spaces. Although this is an interesting result, both the antibody-type and receptor fusion drugs bind to mTNF, so it cannot explain differences in reactivation rates observed for the two drug types. Second, our results suggest three factors: differences in blood concentrations of drugs, TNF/drug binding and unbinding kinetics, and the level of drug permeability into lung tissue can each dramatically affect the likelihood of TB reactivation. In fact, we find that these factors result in different rates of TB reactivation between antibody-type drugs (e.g., infliximab) and TNF receptor fusion proteins (etanercept). Our experimental data from a mouse model suggested that retention of drug concentration in a granuloma as well as the dissociation constant of both antibody and soluble receptors differed. In particular, the presence of high levels of TNF receptors in the granuloma competes for TNF that is temporarily not bound to drugs. Our finding that this occurs with soluble receptor drugs at a much higher level than antibody-based drugs may be involved in the differential effects of these drugs on control of established infection [91]. Finally, although there are differences in drug abilities to induce apoptosis or cytolysis in TNF-expressing key immune cells (e.g., infected and activated macrophages and T cells), our analysis suggests that these activities are not as important as other factors in driving TB reactivation. These findings suggest the characteristics of suitable anti-TNF drugs for treatment of inflammatory diseases while balancing high risks of TB reactivation.

3.4 What Is the Impact of Lymph Node Processes on Granuloma Formation and Function in the Lung?

A key step to mounting a protective immune response to most bacterial infections is effective CD4+ and CD8+ T cell priming in LNs. For TB, it remains unclear how

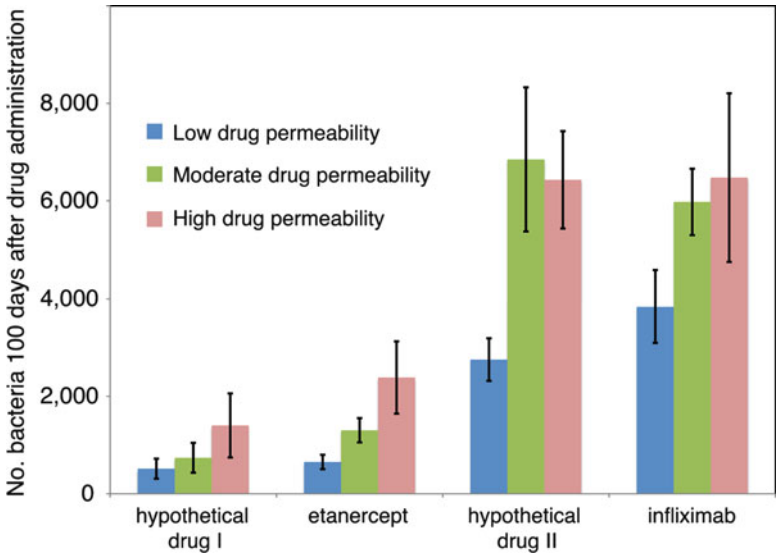

Fig. 7.7 Prediction of the impact of different types of TNF inhibitors on the outcome of *M. tuberculosis* infection at the granuloma scale. 100 days after *M. tuberculosis* infection, at which time a well-circumscribed granuloma with stable bacterial levels ($<10^3$ total bacteria) forms, the granuloma is exposed to one of the TNF-neutralizing drugs. Simulation results (bacterial levels within granulomas at 100 days after treatment with TNF inhibitor) are compared for four different drugs at different levels of drug permeability from vascular sources into lung tissue. Low, moderate, and high permeabilities represent ~10%, ~24%, and ~50% drug permeabilities into lung tissue, respectively. The hypothetical drug I is a drug defined here to only bind sTNF with a TNF:drug binding ratio of 1:1 and TNF binding/unbinding kinetics similar to infliximab. Etanercept binds both sTNF and mTNF with a TNF:drug binding ratio of 1:1. Infliximab binds both sTNF and mTNF and is assumed to have a TNF:drug binding ratio of 1:3 and can induce apoptosis and cytolysis as a result of mTNF binding and cross-linking. The hypothetical drug II is infliximab without apoptotic and cytolytic activities. TNF binding kinetic parameter values and blood concentrations of etanercept and infliximab were used as reported in [104, 105]

events occurring within LNs impact granuloma formation and maintenance. Our recent ABM studies [45–47] emphasize the critical role of T cell related mechanisms in infection progression, such as T cell movement, as well as the magnitude and timing of T cell recruitment. However, mechanistic descriptions of priming, differentiation, and recruitment of immune cells are only partially addressed in these ABM formulations, since these events occur primarily within LNs. We recently took a multi-organ (multi-compartment) approach and built onto the existing agent-based multi-scale model of the lung (described above) some of the main mechanisms of DC and T cell trafficking, as well as T cell priming and differentiation occurring in the lung-draining LN. We described the cellular dynamics occurring within a LN by a DE system, based on a simplified version of the LN compartment portion of our published two-compartmental ordinary differential equation (ODE) model [59]. Our new LN-ODE module tracks the dynamics of antigen presenting cells (APCs, defined as the sum of infected and chronically infected macrophages migrating from the lung

ABM) and several subpopulations of T cells (naïve, precursor, and effector CD4+ and CD8+ T cells). The actions and interactions included are shown in Table 7.1. The magnitude and timing of infection in the lung compartment (generated by the ABM) drive the extent of T cell priming in the LN-ODE model. Effector immune cells are generated in the LN compartment, migrate via blood to the lung, and are input onto the lung. The final result is a *hybrid* multi-compartment mathematical/computational model, where the lung (i.e., granuloma) compartment is described by a discrete/stochastic ABM module, and the LN compartment is represented by continuous/deterministic ODEs. Information is exchanged between the two compartments at every time step. One of the main goals of this work was to investigate how immune mechanisms occurring in the LN impact infection outcomes in the lung, both before and after a granuloma is established. The hybrid model recapitulates typical infection outcomes and predicts biologically relevant cell and bacterial numbers for containment and dissemination scenarios (similar to Fig. 3). Below we review two model predictions that could be relevant to vaccination and immunotherapy strategies.

3.4.1 Prediction I: Antigen Presenting Cell Migration and Immunogenicity Are Key Regulatory Mechanisms in TB Granuloma Formation and Maintenance

Whether the regulation of APC trafficking controls the nature of adaptive immune responses in the lung and in granulomatous tissue in vivo [92] is still an open question. For example, mechanisms governing pulmonary APC trafficking to LNs are still poorly understood, both at cellular and molecular scales. Another complication is that there are currently no assays that directly analyze APCs transiting through lymphatic vessels [93]. We were able to begin to address these questions using our hybrid model described above [57] and our results predicted that the rate of APC trafficking from lung to LN or T cell trafficking from LN to lung can drive the system to either clearance (both before or after a granuloma has been established; see Fig. 7.8) or dissemination and uncontrolled growth of bacteria. Enhancing APC migration is predicted to be a key regulatory mechanism that could be exploited for effective vaccination and immunotherapy strategies. Another prediction of the hybrid model is based on manipulating the efficiency of APC-T cell contacts in vivo (rather than the number of APC migrating to the LN). For example, increasing the duration of the DC–T cell interaction [94, 95], the cognate frequency of naïve T cells [96, 97] or the immunogenicity of DC [98] can all represent viable strategies to clear an infection before a granuloma is fully developed.

3.4.2 Prediction II: Differential Roles of Effector Lymphocytes in TB Containment and Clearance

Our hybrid model implementation confirms an essential role for effector T cells in a successful initial immune response to *M. tuberculosis* invasion: IFN-γ and TNF induce

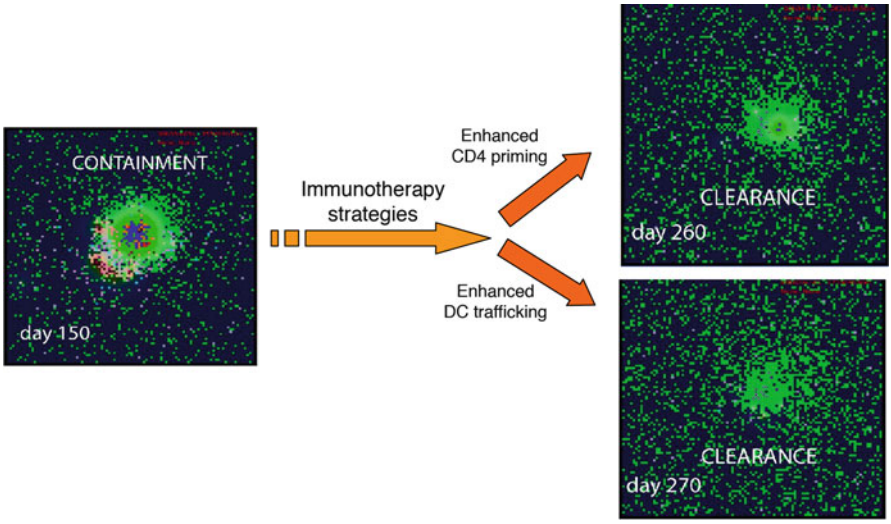

Fig. 7.8 Predictions of the hybrid lung-LN model describing granuloma development and TB infection in the presence of immunotherapy strategies. We only show the effect of enhancing CD4+ T cell priming and DC trafficking to the LN upon bacterial uptake. The snapshots capture granuloma state at different days after initiation of immunotherapy. The initial conditions used yield a typical containment scenario at day 150 post-infection. It takes approximately 10–15 days to resolve the inflammation after bacteria are cleared

macrophage activation that allows the formation of a stable granuloma (containment). In particular, we found that once a granuloma is fully formed, a viable immunotherapy strategy to clear infection in a latently infected host is to specifically enhance effector CD4+ T cell differentiation (see Fig. 7.8). We predict that effector CD8+ T cell cytotoxic activity is important to controlling the onset of infection and possibly for clearance, but has no key role when a granuloma has already been established. This follows since, once a granuloma is fully formed, it is difficult for cytotoxic T cells to reach the center of a granuloma to physically interact with infected and chronically infected macrophages due to crowding effects. Bacterial clearance is better achieved by macrophage activation, which is strictly dependent on TNF and IFN-γ secreting lymphocytes (i.e., CD+ T cells): a successful interaction between effector CD4+ T cells and resting macrophages in the outer layers of a granuloma (in the lymphocyte cuff) is a more viable strategy to combat TB once a granuloma is already established.

4 Conclusions and Future Directions

The granuloma is where the central battle in TB plays out and we believe it reflects the infection status. Thus far our systems biology approach has generated predictions and novel hypotheses regarding cellular and molecular mechanisms influencing granuloma formation and function over a time period of days to years.

Despite years of scientific research and efforts by world health organizations, TB remains a global health problem and is responsible for ~2 million deaths per year. Of great concern is that TB persists as a latent infection in ~2 billion humans worldwide, providing a reservoir of potential disease and contagion. Drug-susceptible TB can be treated only with a lengthy regimen that is fraught with compliance and drug toxicity issues. Drug-resistant TB is a major problem worldwide and development of new drugs and strategies is essential to prevent further spread of these strains. Single drug therapy is not permitted in treatment of human active TB, because drug resistance can arise, and the standard of care must be adhered to. Thus it is difficult to evaluate the effects of new TB drugs or strategies in human clinical trials. There is a critical need for novel approaches and platforms for testing and optimizing new therapies for TB.

Can we use systems biology approaches, particularly those focused on the granuloma, to identify new vaccines or therapeutic strategies for this ancient disease? We believe the field is poised to do just that. For example, combining immune modulation ("immunomodulation") with antibiotics is a potential strategy for enhancing treatment of TB [99, 100]. Several strategies have been tried in murine models (reviewed in [101, 102]) and a few in humans [101, 103], but the results are inconclusive. Appropriate delivery to granulomas and proper timing, drug combinations, and dosing are all likely to be key factors in a successful therapy, but these are difficult to study in mammalian systems due to cost, technical, and ethical issues. A computational platform such as described here could allow for development of various strategies that could then be tested in animal models.

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