CHAPTER 5

MATHEMATICAL MODELS OF ANTI-TNF THERAPIES AND THEIR CORRELATION WITH TUBERCULOSIS

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1 INTRODUCTION

In the last 12 years, different types of drugs that neutralize the proinflammatory molecule tumor necrosis factor alpha (TNFα or, simply, TNF) have been approved by the U.S. Food and Drug Administration (FDA) to treat patients with inflammatory diseases such as rheumatoid arthritis and Crohn's disease [1–3]. Not surprisingly, interfering with TNF, an important cytokine that participates in many immune responses, could affect the immunogenesis of some infectious diseases and ultimately affect the susceptibility of these patients. The best example of an unfortunate side effect is the disease tuberculosis (TB). Following inhalation of *Mycobacterium tuberculosis*, the causative agent of TB, individuals either clear the infection, are able to control but not clear it (known as latent infection), or are unable to control infection and experience active primary disease. Those who have a latent infection have a 10% lifetime chance of reactivation to active disease, usually occurring

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as a result of waning immunity, immunosuppression, or HIV-1/AIDS coinfection. Some patients who had latent TB (who were either aware or not aware of their TB status) underwent anti-TNF therapies for chronic inflammatory illness. A portion of these patients suffered reactivation of latent TB, especially with one drug type [4,5].

In this chapter we describe how mathematical and computational modeling approaches offer useful tools to study mechanisms of action of anti-TNF drugs and how they can interfere with preexisting conditions such as latent TB. Through using these quantitative approaches, we show that it is possible to gain insights into immunological and drug-related factors that might explain the experimental finding of differential reactivation risks for different drugs. We review basic epidemiology and immunology of TB with an emphasis on TNF biology and its role in TB, as well as some details of anti-TNF drugs and their impact on TB disease progression. Finally, we briefly illustrate two mathematical model implementations that capture the immunodynamics of *M. tuberculosis* infection and anti-TNF treatments. The focus is on TNF temporal and spatial dynamics in the presence of anti-TNF drugs.

2 TUBERCULOSIS, TNF, AND ANTI-TNF DRUGS

2.1 Epidemiology of Tuberculosis

TB is a leading cause of death due to infectious disease in the world today, with approximately 8 millions deaths in 2008 and almost one-third of the world population currently infected. *Mycobacterium tuberculosis* (Mtb) is an extraordinary successful bacterium; very few bacilli are sufficient to establish an infection in the lung and are usually able to persist within the host for a long time, sometimes for a person's lifetime, typically without clinical symptoms. Once a person is infected, the most common outcome is latency: the immune response is able to contain the infection through the formation of granulomas: spherical, self-organized cell clusters surrounding bacteria and infected cells. Primary TB develops when the immune response is not successful in containing the initial infection (e.g., if there are deficiencies in the host immune system or if the strain of Mtb is particularly virulent); this infection outcome is characterized by an uncontrolled bacterial growth and dissemination to other organs through the blood. These individuals are also highly contagious, and bacteria are transmitted from airways via coughing, talking, singing, and so on. Long courses (9 to 12 months) of multiple antibiotics (two or more) are required to treat latent and primary TB. If not treated, primary TB leads to death. Reactivation can occur when latent TB is altered and properly formed granulomas are disrupted; bacteria start to proliferate without control and, as in primary TB, if infection is not treated, it disseminates to other organs and causes death. Considering the large number of latently infected individuals (estimated to be approximately 2 billion people), reactivation of latent TB poses a worldwide threat.

2.2 TB Immunology and the Role of TNF

The hallmark of TB infection is the formation of granulomas in the lung tissue. Classical TB granulomas form as organized spherical immune structures composed predominantly of uninfected macrophages surrounding a core of bacteria and infected and activated macrophages with effector T cells localized at the periphery [6,7]. Granulomas physically contain and immunologically restrain bacteria that cannot be cleared. The mechanisms by which granulomas form properly are not completely understood. We know that a key immune factor that helps the host control infection and maintain latency is TNF. Mouse data have shown that neutralizing TNF or its receptors (either during initial infection or during a chronic persistent infection phase) leads to delayed granuloma formation, necrosis, diffuse infiltration, and increased bacterial burden [8–10]. Similar results have been shown in data from nonhuman primates (NHPs), which provide the only established animal model of latent infection [11,12].

2.3 TNF Biology

TNF is produced primarily by macrophages upon infection or exposure to bacteria or bacterial products [13]. Other cell types producing TNF include T cells and natural killer (NK) cells, predominantly in response to chemokines or cytokines (CCL3, IL-1) as well as general cellular stress factors [14]. TNF has four main actions during TB [15]. TNF plays a key role in (1) activation of macrophages, affecting their phagocytic and killing abilities [16,17]; (2) recruitment of many inflammatory cells (key for proper granuloma formation) [18,19]; (3) induction of cytokine and chemokine production [19,20]; and (4) induction of apoptosis of macrophages and T cells [21].

TNF is initially a transmembrane (or membrane-bound, mTNF) protein that undergoes cleavage by the specific metalloproteinase TNF-converting enzyme (TACE) to form a soluble trimer (sTNF) [22]. All soluble TNF is derived from mTNF by proteolytic cleavage, but not all mTNF is cleaved to generate sTNF [23]. Both forms of TNF function by binding to one of two receptors, TNFR1 (TNFRp55) and TNFR2 (TNFRp75) [24]. TNFR1 is expressed constitutively in most tissues, whereas expression of TNFR2 is highly regulated and is typically found in cells of the immune system [25]. Membrane-bound TNF can itself act as a receptor [26] since it contains a casein kinase I motif; for example, its engagement can be triggered by TNF-specific antibodies [27]. There are two major signaling pathways that can be activated as a result of TNF/TNFR interactions: the caspase-mediated apoptotic pathway and the NF-κB-mediated survival pathway. These signaling pathways are controlled primarily at the level of TNF/TNFR1 interactions [28]. Transmembrane ligands of the TNF superfamily are thought to elicit bidirectional signals called *reverse signaling* [29] (due to the potential receptor-like properties of mTNF, as shown by Ferran et al. [30]). The nature of the reverse signaling is probably stimulatory in T cells [31,32] and inhibitory in monocytes and macrophages (e.g., causing an anergic state

through resistance to bacterial LPS) [33–35]. Monocytes and T-cell data from patients with Crohn's disease suggest that mTNF can also induce apoptotic signals [34,36]. The effect of reverse signaling is generally negligible in healthy individuals, while it seems to be amplified by the presence of anti-TNF antibody treatments (and not TNF-receptor fusion molecules) under chronic inflammatory states. Two main mechanisms are initiated by mTNF binding: (1) activation of the complement cascade (due to a high concentration of antibodies) [37], and (2) apoptosis induced by reverse signaling [34,36]. Data on Crohn's disease support activation of complement cascade, but it is not known if this mechanism is shared among other TNF-related pathologies (such as rheumatoid arthritis and ankylosing spondylitis). The main difference between the two effects is that the complement cascade releases intracellular bacteria into the extracellular domain, whereas apoptosis kills most of the intracellular bacterial load [38,39]. Table 1 lists what is presently known or postulated regarding the effects of sTNF and mTNF binding on macrophages and lymphocytes (see [16] for a review).

TNFR1 and TNFR2 can also be engaged by two other members of the TNF superfamily: lymphotoxin (LT) LTα and LTβ [40,41]. LT is active as a secreted homotrimeric molecule (LT_3, also known as TNFβ) [42] and is produced mainly by lymphocytes (CD4+ T cells), B cells, and NK cells [43]. The specific and likely nonredundant functional activity of $LT\alpha_3$ compared to sTNF is not clearly understood. $LT\alpha_3$ binds to TNFR1 with an affinity similar to sTNF [44], and there is also a 30% homology in the amino acid sequence between the two molecules [45]. Interestingly, the results from $LT\alpha_3$ and LTβ knockout (KO) experiments in mice show delayed granuloma formation, structural lymph node defects [46], delayed macrophage activation, and increased bacterial load [47]. Mice data suggest that LTα KO mice are more susceptible to TB infection [46] and that LTα alone cannot rescue TNF KO mice [8]. LTα probably plays a role in the local organization of the granulomatous response rather than in the activation of either macrophages or lymphocytes. More experimental studies are needed to shed light on the different

TABLE 1 sTNF and mTNF Effects on Lymphocytes and Monocytes/Macrophages

Cell type	sTNF	mTNF
	Cell activation [16,17] (through TNFR1 and TNFR2)	
Monocytes and Macrophages	Cell Apoptosis [16] (through TNFR1)	Reverse Signaling: anergy [32]
	Cell Recruitment [18,19]	Reverse Signaling: apoptosis or cell loss (in pathological states) [33]
	Cell activation [16,58] (through TNFR1 and TNFR2)	
Lymphocytes	Cell Apoptosis [16] (through TNFR1)	Reverse Signaling: activation [30,31]
	Cell Recruitment [18,19]	Reverse Signaling: apoptosis or cell loss (in pathological states) [59]

roles of LTα_3 and LTβ as well as to validate whether reverse signaling is important in vivo.

2.4 Anti-TNF Drugs

Currently licensed TNF inhibitors are either anti-TNF monoclonal antibodies or soluble TNF receptors [48]. *Infliximab* and *adalimumab* are anti-TNF antibodies composed of a human IgG1 constant region, with murine and human variable regions, respectively. *Certolizumab* pegol is a PEGylated humanized anti-TNF Fab′ fragment. *Etanercept* is the only soluble TNF receptor presently in clinical use and is composed of two extracellular domains of human TNF receptor 2 (TNFR2) fused to the Fc fragment of human IgG1. A complete review of these TNF inhibitors is given by Wallis [48].

A systematic and comprehensive comparison of anti-TNF drugs has not been performed to date, especially in human subjects. Recent mouse data show that treatment with anti-TNF antibody during chronic TB (a state unique to the mouse where bacterial persistence develops into a protracted chronic infection, with high bacterial burden) quickly resulted in active TB, while treatment with an etanercept-like molecule (receptor fusion) did not impair control of the infection [49]. Recent data on NHP with latent infection receiving TNF-neutralizing agents [either an inhibitor of soluble TNF, recombinant methionyl human soluble TNF receptor I (p55-TNFRI), or adalimumab] showed a high reactivation rates with no reported significant difference between the two agents [50].

Regarding human data, a surveillance system called the Adverse Event Reporting System (AERS) has been established by the FDA; drug manufacturers are required to submit reports of adverse events and health care professionals, and consumers are encouraged to do so as well. In a 2004 study of granulomatous infections associated with infliximab and etanercept [5] use (reported in AERS), identification of active TB was the most frequently reported adverse reaction, occurring in about 144 per 100,000 patients with infliximab treatment and in about 35 per 100,000 patients with etanercept treatment. The different TB reactivation risks observed also appear significant based on other more recent meta-analysis studies [4,51,52], where the frequency of reactivation of tuberculosis in anti-TNF antibody (i.e., infliximab)–administered patients appears to be higher than for TNF receptor fusion (i.e., etanercept). These differential risks suggest that the mode of action of these agents is different, and that possibly the protocol regimens of anti-TNF therapies could be modified to get better outcomes. Table 2 illustrates some of the details for the two anti-TNF drugs that we focused on earlier: infliximab and etanercept.

Infliximab is a human–mouse chimeric monoclonal TNF antibody. It binds with high affinity to monomeric and trimeric TNF and both soluble and membrane-bound TNF. It does not bind to soluble LTα_3 [53]. Up to three molecules of infliximab can bind each TNF molecule. As a bivalent monoclonal antibody, infliximab can bind two sTNF trimers simultaneously [53,54]. Formation of large immune complexes is possible, especially in the presence of high levels of TNF. Infliximab is known to cross-link mTNF and thereby induce apoptosis or

TABLE 2 Anti-TNF Drugs Modeled

	Anti-TNF Antibody (Infliximab) [60]	TNF Receptor Fusion (Etanercept) [60]
Description	Chimeric monoclonal TNF antibody	TNF receptor p75-IgG fusion protein rather than an antibody
Ligands	Both monomeric and trimeric; both sTNF and mTNF	Only trimeric TNF; both sTNF and mTNF; $LT\alpha_3$, $LT\alpha_2\beta_1$
mTNF binding	Strong	Moderate
TNF-binding stoichiometry	Up to three drug molecules can bind each trimeric TNF molecule; up to two TNF molecules can bind each drug molecule	Drug binds trimeric TNF with a binding ratio of 1 : 1
TNF-binding kinetics	Slower binding and unbinding kinetics, but higher affinity for TNF than for etanercept	Faster binding and unbinding kinetics, but lower affinity for TNF than for infliximab
mTNF cross-linking and inducing apoptosis/CDC	Yes	No
PD–PK	Half-life of 9.5 days	Half-life of 3.5 days
Dose and administration	2 h intravenous infusion RA: 3–10 mg/kg at weeks 0, 2, and 6 and every 4–8 weeks CD: 5 mg/kg at weeks 0, 2, and 6	SC injections RA, PsA, AS: 25 mg biweekly, 50 mg per week JRA: 0.4 mg/kg, 0.8 mg/kg

complement-dependent cytolysis in TNF-expressing cells, including infected and activated macrophages and CD4+ and CD8+ T cells [34,55–58].

Etanercept is a TNF receptor p75-IgG fusion protein. It binds selectively to human trimeric TNF and $LT\alpha_3$ with a binding ratio of 1 : 1 [53,54]. Because etanercept binds mTNF in a 1 : 1 ratio, it is not able to cross-link mTNF and thus does not induce apoptosis or complement-dependent cytolysis [55–58].

3 THEORETICAL MODELS TO STUDY TB INFECTION

Many of the animal studies described earlier cannot be performed in human subjects. NHP models are very similar to humans in almost all aspects [59,60], but are expensive. In an effort to complement experimental studies, we have developed several mathematical and computational models that have helped add to our understanding of immune protection mechanisms, as well as the different roles of TNF in tuberculosis control and pathology. We have used different modeling systems in an attempt to find the optimal approach for addressing different questions [61]: ordinary differential equations (ODEs) [62–66], partial differential

equations (PDEs) [67,69], and agent-based models (ABMs) [15,68,70]. In addition, we developed refined methods for performing uncertainty and sensitivity analysis in each of these settings (Marino et al. [71]). These analysis tools guided our model building, validation and calibration steps, and parameter estimation. Model validation was also performed by replicating in a semiquantitative fashion known experimental results for typical infection scenarios (e.g., bacteria and cell counts in latency and primary TB) from different animal systems, and also by performing virtual TNF gene knockouts and TNF neutralization studies. For example, in mouse and NHP systems TNF or TNFR1 knockouts both lead to uncontrolled growth of Mtb [72–74]; our virtual models recapitulate this phenomenon. In the case of virtual TNF deletion, granulomas that form are greater in size, irregular in structure, and include very high numbers of extracellular Mtb, large numbers of infected macrophages, and widespread dead tissue caused by multiple deaths of macrophages, usually within the core of the granuloma [15,68–70].

Here we review two recent ODE and PDE models that we have developed to focus on TNF immunodynamics during TB infection in the lung, either at a cell or at a molecular level. We then use these TB models to shed some light on mechanisms underlying differential reactivation rates between the two classes of drugs: anti-TNF antibody and TNF receptor fusion.

3.1 ODE Model: TB Reactivation Based on TNF Bioavailability and Fraction of TNF That Is Soluble vs. Membrane-Bound Fraction

We track the temporal dynamics of cytokine concentrations (IFN-γ, IL-12, total TNF, IL-10, and IL-4), bacteria (intracellular and extracellular), macrophage (resting, activated, and infected), and T-cell populations (Th0, Th1, Th2, and CD8+ T-cell subsets) in a system of 16 nonlinear ODEs. Model equations have been developed by Marino et al. [65].

TNF Bioavailability and Reactivation Threshold Starting from a baseline latent TB infection scenario (stable bacterial loads and cytokine levels, as well as most of the immune cell counts), we explore the effects of TNF neutralization induced by a TNF-neutralizing antibody and a soluble p75 TNF receptor fusion molecule by varying several immune or drug-related factors. To quantify these changes systematically, we define *TNF bioavailability* as the amount of TNF available for use in the granuloma during anti-TNF treatment. High (low) TNF bioavailability during treatment translates into low (high) neutralization power of the drug. Since we model TNF concentrations in granulomatous tissues, we can interpret *TNF bioavailability* alternatively in terms of drug penetration: High (low) *TNF bioavailability* during treatment translates into high (low) tissue penetration of the drug into granulomatous tissues. In vivo data on drug neutralization power are not available; each drug may have a different capability of neutralizing TNF. We captured drug neutralization power by decreasing the total level of TNF achieved during latency by a certain percentage. So, for example, a drug neutralization power of 80% means that the drug is able to neutralize 80% of the bioavailable TNF

during latency; thus, the *TNF bioavailability* during anti-TNF treatment is only 20% (of the latency level). The model has a single equation for total TNF, labeled Fα. Soluble TNF (sTNF) is represented by σFα, where the parameter σ indicates the fraction of TNF cleaved and released in soluble form; thus, mTNF $= (1 - \sigma)$Fα. The effects of soluble and membrane-bound TNF on different cell populations (as described in Table 1) are captured in the model. Under pathological conditions (chronic inflammatory states), the presence of anti-TNF antibodies (and not TNF-receptor fusion molecules) and subsequent binding to mTNF can induce activation of the complement cascade (due to high antibody concentrations) [37] and cell loss induced by reverse signaling through mTNF binding [34]. We include both of these effects in the model. We do not directly include LTα in the model, but we account for LTα-dependent recruitment of macrophages and lymphocytes by down-regulating all TNF-independent recruitment terms during receptor fusion treatment simulations. We also define the concept of *reactivation threshold* (RT) as the level (% of TNF in latency) of bioavailable TNF below which the system reactivates TB. Proxy for reactivation is the uncontrolled bacterial growth and dissemination. Figure 1 shows how we implemented the concepts of TNF bioavailability and reactivation threshold in the ODE model.

Virtual Clinical Trials to Mimic the Effects of Anti-TNF Therapies on Virtual TB Patients In order to compare the effects of two types of anti-TNF drugs, we performed several virtual clinical trials, where we vary, either alone or in combinations, the fraction σ of TNF cleaved and released in the soluble form, and TNF bioavailability during anti-TNF treatment. To capture biological variation between patients, we use a Latin hypercube sampling scheme (see [71] for a review) to test combinations of TNF bioavailability and fraction σ of soluble TNF within the ranges 0 to 100% and 50 to 100%, respectively. The sample size used is 100. Anti-TNF treatments are implemented following the protocols described in Table 2. The ODE model predicts that bioavailability of TNF following anti-TNF therapy is the primary factor inducing reactivation of latent infection. Reactivation of latent TB always occurs if both drugs penetrate the granuloma equally well (TNF bioavailability less than 20%). Poor drug penetration (TNF bioavailability $>$50%) yields no reactivation occurrences for either drug type. We found that infliximab outnumbers etanercept in reactivation cases (up to a ratio of 8 to 1) when TNF bioavailabilities are within the range 20 to 50% of the TNF latency levels. We also found that mTNF plays a relevant role in TB reactivation during anti-TNF therapy. We predict that down-regulating membrane-bound TNF cleavage (i.e., lower fraction σ of soluble TNF) has a negative impact on maintaining latency during anti-TNF antibody treatment compared to the receptor fusion molecule, with significantly higher reactivation thresholds (28.62% vs. 25.01%, $p < 0.001$).

The probability of TB reactivation increases with higher bacterial load at treatment initiation, suggesting that a complete regimen of antibiotic treatment for *M. tuberculosis* infection prior to anti-TNF treatment could reduce the risk of reactivation. If we assume that both drugs have similar TNF neutralization power, results from sensitivity analysis suggest that differential cell loss rates and levels induced by apoptosis from anti-TNF antibody therapies could account for

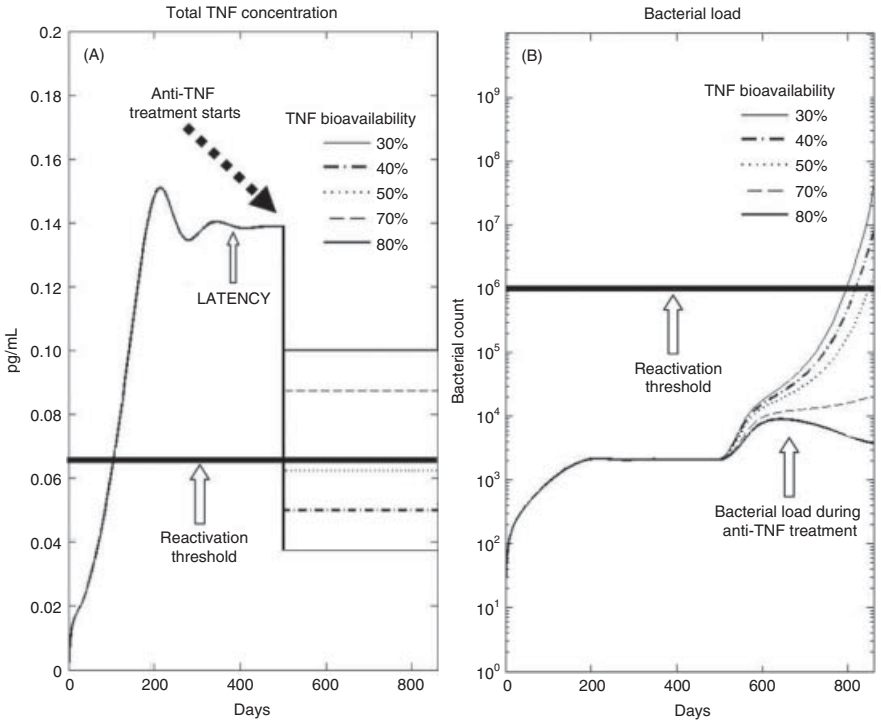

FIGURE 1 TNF bioavailability and reactivation threshold: implementation of anti-TNF treatments into the ODE model described in Section 3. Temporal plots of total TNF concentrations (A) and corresponding bacterial loads (B) for a latency scenario (from day 0 to day 500) and after a hypothetical anti-TNF treatment affects TNF bioavailability (from day 500 on) by a fixed percentage. The x-axis shows day post infection and post anti-TNF treatment, and the y-axis represents TNF concentrations in pg/mL (A) and bacterial counts (B). A reactivation level (B) is set to 10^6; any trajectory beyond that level is classified as reactivation. TNF bioavailability is varied from 30 to 80% of the latency level.

higher reactivation rates; loss of effector lymphocytes (CD8+ T cells) and activated macrophages impairs immune protection and enhances bacterial dissemination.

3.2 PDE Model: TNF Receptor Dynamics and Cellular Organization in a Tuberculosis Granuloma

As a second example of how models can be useful for predicting the impact of drugs, we describe a reaction/diffusion-based PDE model that studies the impact of TNF receptor (TNFR) dynamics on the bioavailability of TNF in a TB granuloma [69]. This model is based on a simple experimental system for granuloma formation in mice. An induced granuloma forms in mice following injection of Sepharose beads covalently coupled to *Mycobacterium*-purified protein derivative (PPD) antigen [75,76]. We developed a mathematical model that considers a simple representation of the spatial structure of a granuloma. It captures a granuloma as

a distinct continuous collection of immune cells forming concentric layers. It also includes molecular-level details regarding TNF/TNFR binding and trafficking processes (defined here to include synthesis, internalization, recycling, and degradation of ligand and receptors). TNF/TNFR kinetic parameter values were either estimated from the literature or measured in the experimental mouse model described above. In this simplified model, there is no readout for bacterial levels; the PDE model is focused on TNF/TNFR-level reactions and interactions while using a coarse-grain description of the cellular-level details representing a single snapshot in time of a granuloma comprised of a static number of immune cells. The mathematical model also accounts for a bead at the center of the granuloma as is used in the mice experiments. We use the mathematical model to identify key processes controlling TNF concentration in a granuloma. In particular, this PDE model allows the study of how the spatial organization of immune cells within a granuloma (i.e., a core of macrophages surrounded by a mantle of T cells) can affect the amount of TNF available for signaling for different granuloma cells. Using our model containing molecular-scale details, we also studied how TNF-binding properties of TNF-neutralizing drugs influence their neutralization power.

Single-Cell TNF/TNFR Kinetics and Cellular Organization Within a Simulated Bead Granuloma Binding interactions and reactions controlling the single-cell-level TNF/TNFR dynamics are illustrated in Figure 2A. The details of the model reactions and equations are given by Fallahi-Sichani et al. [69]. Here we briefly review some key mechanisms included in the model. As described in the TNF biology section, TNF-producing cells synthesize and express TNF as a membrane-bound precursor form (mTNF) that can then be processed and released as a soluble form (sTNF) into extracellular spaces. This processing occurs via TACE [42,77]. Two types of TNF receptors (TNFR1 and TNFR2) are synthesized and expressed on the cell surface as free receptors. sTNF binds reversibly to TNFRs on the cell membrane or degrades [78–80]. sTNF-bound cell surface TNFR1 internalizes and sTNF-bound cell surface TNFR2 may undergo internalization or shedding into extracellular spaces [81].

Internalized receptors may degrade or recycle to the cell membrane, where they can rebind to sTNF [34]. Ligand-free TNFRs also turn over (internalize) [82,83]. In the extracellular space, intact sTNF may dissociate from the sTNF/TNFR2 complex shed. [84]. Molecular processes described above are incorporated into the coarse-grain multicellular static model of a bead granuloma (Fig. 2B). Within this multiscale model, TNF is produced by TNF-producing immune cells (i.e., macrophages as identified via experiments [69]), diffuses into extracellular spaces, and interacts with TNFRs on the membranes of cells. To study the impact of spatial organization of immune cells, the bead granuloma is modeled as a spherical continuum consisting of two cellular compartments. The inner compartment includes a large number of macrophages that form the core of the granuloma, and the outer compartment or mantle is comprised primarily of T and B cells (Fig. 2B). This is consistent with the structures observed for classical TB granulomas [6,7]. We define a metric, *separation index, s*, representing the level of separation between different cell types in a granuloma (i.e., how well mixed the

granuloma mantle and core are for different types of cells). The separation index is defined as $s = (l_o - l_g)/(1 - l_g)$, where l_o and l_g are the lymphocyte (T cell and B cell) fractions in the outer compartment and in the whole granuloma, respectively.

Thus, a separation index of 1 (see Fig. 2C) represents a separate cellular organization between mantle and core (as observed in human and NHP models of TB), whereas a separation index of zero (see Fig. 2E) is equivalent to a totally mixed cellular organization. Details on the PDE model equations have been provided by Fallahi-Sichani et al. [69]. The model can be used to predict the free sTNF

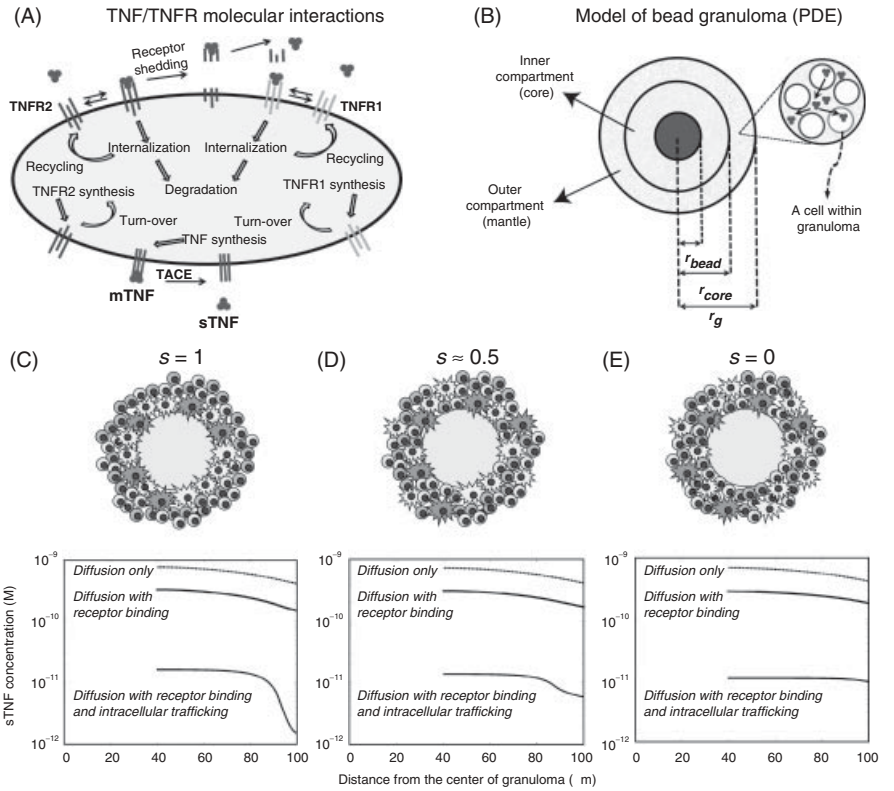

FIGURE 2 Schematic representation of (A) molecular-scale reactions and interactions controlling TNF/TNFR dynamics at the single-cell level, (B) the two-compartment model of PPD bead granuloma, and (C)–(E) model predictions for the steady-state spatial distribution of free sTNF within a bead granuloma for various separation index (s) values. The two-compartment model of granuloma includes a bead of radius r_{bead} (the radius is 40 micrometer, as shown in the plots, where the sTNF concentrations always start at 40 micrometers) surrounded by the inner compartment populated by macrophages and dendritic cells and the outer compartment concentrated by lymphocytes. Receptor binding and intracellular trafficking of TNF significantly reduce the steady-state concentration of sTNF within a granuloma in comparison with the case that TNF diffuses in extracellular spaces without binding to cell-surface TNFRs. Greater separation indices in the presence of TNF/TNFR intracellular trafficking lead to steeper sTNF gradients within a granuloma.

concentration as a function of radial position in the granuloma; other concentrations (e.g., bound TNFR1) are also predicted.

TNF/TNFR Binding, Trafficking Dynamics, and Cellular Organization Control TNF Bioavailability Within a Granuloma Analyzing our model, we characterize two important mechanisms for controlling the steady-state concentration of TNF within a granuloma. One of these mechanisms is the specific spatial organization of immune cells within a granuloma (i.e., the level of separation between different classes of cells). Further, intracellular trafficking of TNF via internalization of recyclable TNFRs enhances the impact of cellular organization on spatial distribution of available TNF in a granuloma (Fig. 2C–E). Indeed, the specific organization of different cell types in a granuloma core and mantle induces a spatial heterogeneity in the level of TNF expression and TNFR densities and thus the amount of internalized TNF within a granuloma. This induces a *gradient of TNF* within a granuloma that affects the amount of TNF available for signaling for each specific cell type. More recent work on this topic was performed by our group and confirms that indeed the gradient is relelvant [108]. For sufficiently large values of the separation index (s) in a granuloma, the model predicts significantly greater concentrations of sTNF in the macrophage-rich core than in the lymphocyte-rich mantle (e.g., Fig. 2C). We suggest that such a TNF gradient might be important for spatially coordinating the TNF-induced immunological functions in a granuloma. In particular, a key difference between the two major signaling pathways controlled primarily at the level of TNF/TNFR1 interactions is that the NF-κB pathway, in contrast to the apoptotic pathway, can generally be activated by very low concentrations of TNF (i.e., less than 10^{-11} M and as low as 10^{-13} M) that can activate only a limited number of cell-surface TNFR1s [85–88]. Thus, the TNF gradient in a granuloma may lead to a differential induction of apoptotic and survival signaling pathways between the granuloma core and the surrounding ring of lymphocytes of classical granulomas with large separation indices (such as those observed particularly in humans, as well as NHP and guinea pig models of TB [89]). In other words, steep TNF gradients within a granuloma lead to higher levels of apoptosis of macrophages in the core of granuloma than in lymphocytes in the mantle. This hypothesis has immunological implications. Infected macrophages are generally located in the core of granulomas. Thus, TNF-induced apoptosis of infected macrophages may aid in antigen cross-presentation and subsequent T-cell priming and help eliminate the pathogen [90,91]. Survival of T cells surrounding the macrophages allows them to function to activate macrophages to kill bacteria [92]. Thus, a separate cellular organization in a granuloma may favor an efficient immune response via spatially coordinating the TNF-induced immunological functions. In current work we are testing this concept of a cytokine gradient experimentally (data not shown).

Simulation of TNF Neutralization in a Bead Granuloma Using the PDE model above, we simulate addition of a drug molecule as an agent that binds to sTNF, or to both sTNF and mTNF molecules, and also inhibits sTNF binding to both TNFRs. TNF neutralization-associated reactions in the model include reversible binding of drug to mTNF and sTNF [53,57], release of drug-bound

mTNF into extracellular spaces due to TACE activity, and drug or TNF–drug complex degradation [93]. TNF neutralization-associated reactions and equations are given by Fallahi-Sichani [69]. To study the effect of TNF-neutralizing drugs on TNF bioavailability in a granuloma, the model is simulated in the absence of drug until a steady state is reached, and then simulation of addition of drug occurs. Similar to the TNF bioavailability concept introduced in the ODE model, we define the *TNF neutralization efficiency*, E, as the ratio of the spatially averaged steady-state concentration of sTNF before addition of drug to the spatially averaged concentration of sTNF when drug exerts its maximum effect (i.e., approximately 6 h after drug addition). We use reported average serum concentration of two murine analogs of human TNF blockers infliximab and etanercept (both within the order of 10^{-7} to 10^{-6} M, after a single dose [94]) to estimate the tissue-level concentration of these drugs.

We modeled three classes of hypothetical drugs based on properties of human TNF-neutralizing drugs (e.g., infliximab and etanercept), and their efficiencies of TNF neutralization are compared. A class 1 drug is defined to bind sTNF, but not mTNF, at a binding ratio of 1 : 1; a class 2 drug binds both sTNF and mTNF at a binding ratio of 1 : 1; and a class 3 TNF-neutralizing drug binds both sTNF and mTNF at a TNF/drug binding ratio of 1 : 3. The possibility of the higher binding ratio for a class 3 drug results from both sTNF and mTNF being trimeric in their mature bioactive form [95]. A class 3 drug may have more than one binding site for TNF, allowing formation of larger drug–TNF complexes (not captured in the model). A sTNF molecule with either one, two, or three drug molecules bound is neutralized and not able to bind TNFR1 or TNFR2. This assumption is consistent with experimental data indicating that only trimeric TNF is biologically active and that both monomeric TNF and artificially prepared dimeric TNF do not trigger signaling in cells efficiently [96,97].

Figure 3 shows a comparison of the TNF neutralization efficiency (E) by drugs of classes 1 to 3. We use TNF association and dissociation kinetics of the two major human TNF blockers, infliximab and etanercept, for comparison. These results suggest how the efficiency of TNF neutralization within a granuloma depends not only on the affinity of the drug for TNF, but also on the ability of the drug to bind to mTNF versus sTNF, the rate constants for drug–TNF association and dissociation reactions, as well as the drug–TNF binding stoichiometry. TNF–infliximab and TNF–etanercept association–dissociation kinetics for a class 1 drug lead to neutralization efficiencies of less than 10% in a bead granuloma. However, at all values of TNF–drug association and dissociation rate constants, a drug with the ability to bind to both sTNF and mTNF (classes 2 and 3) is more efficient in neutralizing TNF than is a drug that can only bind to sTNF (class 1). This demonstrates that even if sTNF is considered to be the primary form of TNF that controls TNF-mediated signaling in granuloma cells, mTNF binding is an important determinant of neutralization power of TNF-neutralizing drugs. Our simulations also show that at TNF–drug association and dissociation kinetics of etanercept and infliximab, a higher drug–TNF binding ratio (i.e., 3 : 1) of a class 3 drug slightly increases the efficiency of TNF neutralization compared to a drug of class 2 type with a binding ratio of 1 : 1.

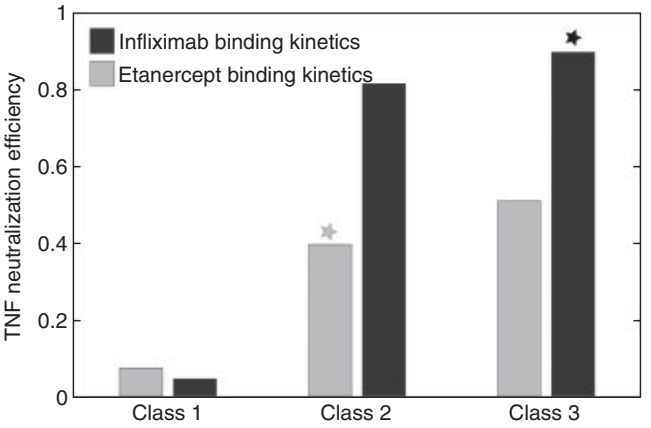

FIGURE 3 Model predictions for the effect of TNF-neutralizing drugs of different class types on the bioavailability of TNF within a bead granuloma, using TNF–drug association and dissociation kinetics of the human TNF blockers etanercept and infliximab. Class 1: the drug can only bind to sTNF with a binding ratio of 1 : 1; class 2: the drug can bind to both mTNF and sTNF with a binding ratio of 1 : 1; and class 3: the drug can bind to both mTNF and sTNF with a drug/TNF binding ratio of 3 : 1. The black and grey stars indicate predicted neutralization efficiencies for etanercept and infliximab, respectively.

Further, the model compares the neutralizing power of infliximab and etanercept, based on their TNF binding specificities and class type. Infliximab can be estimated as a class 3 drug, as up to three infliximab molecules can bind to one trimeric mTNF or sTNF molecule. Etanercept can be considered a class 2 drug, as it can bind to both trimeric sTNF and mTNF with a binding molar ratio of 1 : 1 [53,98]. Our bead granuloma model predicts TNF neutralization efficiencies of 0.90 and 0.39 for drugs with identical TNF binding properties to infliximab and etanercept, respectively; these efficiencies are marked with stars on Figure 3. If we apply these TNF neutralization efficiencies to the virtual clinical trial results of the ODE model described earlier, infliximab treatment will have a 100% reactivation rate, whereas etanercept will not reactivate a single patient. Thus, the difference in binding properties of infliximab and etanercept must be considered when explaining the higher rate of TB reactivation induced from infliximab treatments than from etanercept, although differential tissue permeabilities of drugs [49] as well as functional properties such as induction of apoptosis in TNF-producing cells by infliximab but not etanercept may further influence the outcome of anti-TNF treatments [47,99].

4 PRESENT AND FUTURE WORK

Many questions in TB immunobiology are still incompletely understood. For example, which immune factors are responsible for determining different infection outcomes, how these immune factors interact within the granuloma, and how antibiotics and immunomodulators such as anti-TNF drugs might affect a

protective host immune response. Experimental and modeling data support a key role for TNF. We show how TNF-associated processes and ultimately the outcome of Mtb infection can be affected by TNF concentration gradients. However no experiments have yet been done to explore the main factors driving TNF concentration during TB granuloma formation and maintenance in vivo and if a TNF gradient exists in vivo. We could potentially target TNF-level processes that might develop into new strategies for disease treatment and complement antibiotic therapies.

Our efforts have been focused on developing quantitative approaches to reconstruct and integrate known mechanisms driving the immune response to TB and granuloma formation into a mathematical and computational platform. Recently, we have developed multiscale computational models (i.e., agent-based models) that describe the temporal and spatial dynamics of the immune response to Mtb in the lung, with the emphasis on granuloma formation and maintenance [15,68,70]. The last study [68] recapitulates TB granuloma formation and maintenance over three biological length scales [68]: molecular, cellular, and tissue. Cellular- and tissue-scale dynamics are captured via a set of well-described interactions (rules) between immune cells and the pathogen (Mtb), while single-cell molecular-scale processes that control TNF/TNFR binding and trafficking for each individual cell (as described in Fig. 2A) are captured by a set of nonlinear ODEs. These molecular and cellular scales communicate with each other by two major TNF-induced signaling pathways: caspase-mediated apoptosis and NF-κB activation, which are both controlled at the level of TNF/TNFR1. The NF-κB signaling pathway is initiated by TNF-bound cell-surface TNFR1, while apoptosis depends on the internalized sTNF/TNFR1 complexes [100–102].

The cellular–tissue scale captures basic processes, such as chemotactic movement and recruitment of immune cells to site of infection, intracellular and extracellular growth of Mtb, phagocytosis of bacteria by macrophages, cell death and apoptosis, macrophage/T-cell interactions, down-regulation of immune cells by regulatory T cells, secretion of chemokines, and more. Details on the rules and on the implementation have been given by Fallahi-Sichani et al. [68].

This ABM implementation replicates typical infection outcomes in TB, such as the containment scenario, where a stable solid granuloma with caseous center emerges [73,103] and coexistence between Mtb and the host is established and maintained. Other possible granuloma outcomes can also be replicated (e.g., clearance and dissemination) by manipulating values of important model parameters. A current application of this multiscale modeling platform investigates mechanisms underlying tuberculosis reactivation induced by anti-TNF therapies [108]. Since the ABM predicts spatial and temporal dynamics of TNF during development of a granuloma in lung tissue (data currently not measurable experimentally), we can use this multiscale computational platform to investigate mechanisms by which hypothetical TNF-neutralizing drugs that diffuse in TB lesions may interfere with immune response and reactivate TB.

We know many of the drug properties, such as (1) TNF/drug-binding affinity and kinetics, stoichiometry, and drug ability to bind membrane-bound TNF (mTNF); (2) drug permeability from blood vessels into lung tissue; (3) drug ability

to stimulate apoptosis or cytolysis of key immune cells; and (4) drug pharmacokinetic characteristics. Structural and functional properties of the drugs have led the scientific community to formulate various as-yet-untested hypotheses regarding their mechanisms of action in reactivating TB [47,48,104–107]. One of the major limitations is that a comprehensive experimental analysis of the effect of each of these drug characteristics on the immune response to Mtb, alone and in combination, is at present very difficult. Our recent study [108] suggests that drug binding to membrane-bound TNF critically impairs granuloma function. The model also suggests that a higher risk of reactivation induced from antibody-type treatments is primarily due to differences in TNF/drug binding kinetics and permeability. Apoptotic and cytolytic activities of antibodies and pharmacokinetic fluctuations in blood concentration of drug seem to be not essential to inducing TB reactivation.

A multiscale mathematical–computational modeling approach such as the one just highlighted can complement experimental studies aimed at discovering immune factors that are central to infection control in a granuloma in the presence of TNF inhibitors in vivo, as well as in identifying functional and biochemical characteristics underlying the higher potency of some TNF-neutralizing drugs in reactivating TB compared to other drugs. Results of such studies can aid in the development of safer anti-TNF drugs and treatment protocols for inflammatory diseases, contributing to lowering the burden of TB reactivation cases and ultimately helping to eradicate TB.

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