Differences in Reactivation of Tuberculosis Induced from Anti-TNF Treatments Are Based on Bioavailability in Granulomatous Tissue

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The immune response to Mycobacterium tuberculosis (Mtb) infection is complex. Experimental evidence has revealed that tumor necrosis factor (TNF) plays a major role in host defense against Mtb in both active and latent phases of infection. TNF-neutralizing drugs used to treat inflammatory disorders have been reported to increase the risk of tuberculosis (TB), in accordance with animal studies. The present study takes a computational approach toward characterizing the role of TNF in protection against the tubercle bacillus in both active and latent infection. We extend our previous mathematical models to investigate the roles and production of soluble (sTNF) and transmembrane TNF (tmTNF). We analyze effects of anti-TNF therapy in virtual clinical trials (VCTs) by simulating two of the most commonly used therapies, anti-TNF antibody and TNF receptor fusion, predicting mechanisms that explain observed differences in TB reactivation rates. The major findings from this study are that bioavailability of TNF following anti-TNF therapy is the primary factor for causing reactivation of latent infection and that sTNF—even at very low levels—is essential for control of infection. Using a mathematical model, it is possible to distinguish mechanisms of action of the anti-TNF treatments and gain insights into the role of TNF in TB control and pathology. Our study suggests that a TNF-modulating agent could be developed that could balance the requirement for reduction of inflammation with the necessity to maintain resistance to infection and microbial diseases. Alternatively, the dose and timing of anti-TNF therapy could be modified. Anti-TNF therapy will likely lead to numerous incidents of primary TB if used in areas where exposure is likely.

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Introduction

Control of Mycobacterium tuberculosis (Mtb) infection is a result of a successful immune response that requires priming and activation of antigen-specific CD4+ and CD8+ T lymphocytes, recruitment of cells to the infection site (typically the lung), and production of cytokines, some of whose role is to activate macrophages. This leads to inhibition or killing of some but not all bacilli. Immunological structures (granulomas) form in the lung in response to persistent antigen and cytokine and chemokine signals. In 95% of infected hosts, M. tuberculosis (Mtb) persists without causing symptoms or disease. Latent infection can subsequently reactivate to cause active TB. Experimental evidence has revealed that tumor necrosis factor (TNF) plays a major role in host defense against Mtb in both the active and chronic phases of infection [1–4].

TNF action increases the phagocytosis by macrophages and enhances mycobacterial killing in concert with IFN-γ [3,5]. TNF is crucial in recruitment of inflammatory cells, stimulating chemokine production [6] and inducing adhesion molecules on vascular endothelium [7].

Table S1 summarizes data regarding TNF in Mtb murine models. TNF is a crucial component of both antibacterial protection and the inflammatory immune response. TNF-deficient mice exhibit disorganized granulomas, altered tissue pathology, high bacterial loads, and reduced survival [2,3]. TNF also possesses tissue-injuring properties that manifest in clinical settings including inflammation, auto-immune diseases, and transplant rejections [8,9]. In TB patients, peripheral increases in TNF have been implicated in clinical worsening [10]. In the absence of TNF signaling, disruption of granulomatous formation as well as dissolution of granulo-

Abbreviations: AERS, Adverse Event Reporting System; LHS, latin hypercube sampling; MA, activated macrophage; MI, infected macrophage; Mtb, Mycobacterium tuberculosis; PRC, partial rank correlation; sTNF, soluble tumor necrosis factor; TB, tuberculosis; tmTNF, transmembrane TNF; TNF, tumor necrosis factor; VCT, virtual clinical trial.

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Tuberculosis (TB) is the leading cause of death due to infectious disease in the world today. It is estimated that 2 billion people are currently infected, and although most people have latent infection, reactivation occurs due to factors such as HIV-1 and aging. Antibiotic treatments exist; however, there is still no cure and the current vaccine has proven to be unreliable. Experimental science has uncovered a plethora of immune factors that help the host control infection and maintain latency. One such factor, tumor necrosis factor alpha (TNF), is a protein that facilitates cell–cell communication during an inflammatory immune response. Animal models have shown that TNF is necessary for control of TB infection. Different types of anti-TNF drugs were developed for patients with non-TB related inflammatory diseases such as rheumatoid arthritis and Crohn's disease. Some of these patients who had latent TB suffered reactivation, especially with one drug type. Because these studies cannot be performed in the mouse, and nonhuman primates are expensive, we developed a computational model to perform virtual clinical trials (VCTs) that predicted why reactivation occurs and why it happens differentially between the two classes of drugs tested. We make recommendations on how this issue can be combated.

Several biologic inhibitors (antibodies and receptor fusion molecules) have been developed that interfere with TNF activity and are used to control inflammation in diseases such as rheumatoid arthritis and Crohn’s disease. Some of these patients who had latent TB suffered reactivation, especially with one drug type. Because these studies cannot be performed in the mouse, and nonhuman primates are expensive, we developed a computational model to perform virtual clinical trials (VCTs) that predicted why reactivation occurs and why it happens differentially between the two classes of drugs tested. We make recommendations on how this issue can be combated.

**Author Summary**

Tuberculosis (TB) is the leading cause of death due to infectious disease in the world today. It is estimated that 2 billion people are currently infected, and although most people have latent infection, reactivation occurs due to factors such as HIV-1 and aging. Antibiotic treatments exist; however, there is still no cure and the current vaccine has proven to be unreliable. Experimental science has uncovered a plethora of immune factors that help the host control infection and maintain latency. One such factor, tumor necrosis factor alpha (TNF), is a protein that facilitates cell–cell communication during an inflammatory immune response. Animal models have shown that TNF is necessary for control of TB infection. Different types of anti-TNF drugs were developed for patients with non-TB related inflammatory diseases such as rheumatoid arthritis and Crohn’s disease. Some of these patients who had latent TB suffered reactivation, especially with one drug type. Because these studies cannot be performed in the mouse, and nonhuman primates are expensive, we developed a computational model to perform virtual clinical trials (VCTs) that predicted why reactivation occurs and why it happens differentially between the two classes of drugs tested. We make recommendations on how this issue can be combated.

TNF is initially a transmembrane (tmTNF) protein that undergoes cleavage by the specific metalloproteinase TNF-converting enzyme (TACE) to form a soluble trimer [soluble tumor necrosis factor (sTNF)] [12]. Both forms of TNF function by binding to one of two receptors, TNFR1 (TNFRp55) and TNFR2 (TNFRp75) [13]. It was reasoned that transmembrane ligands of TNF superfamily might elicit bidirectional signals (reverse signaling) [14]. That hypothesis was supported by data describing potential receptor-like properties of tmTNF [15]. The majority of reverse signaling described in lymphocytes is stimulatory [16,17], whereas monocytes are mainly inhibited in their effector functions [18–20]. For further details, we review TNF biology (sTNF and tmTNF, receptors, reverse signaling, and the role of lymphotoxin) in Text S1. Known effects of sTNF and tmTNF on apoptosis [62] (through TNFR1) Reverse signaling: activation [38] (through TNFR1 and TNFR2) are used to control inflammation in diseases such as rheumatoid arthritis and Crohn’s disease. Some of these patients who had latent TB suffered reactivation, especially with one drug type. Because these studies cannot be performed in the mouse, and nonhuman primates are expensive, we developed a computational model to perform virtual clinical trials (VCTs) that predicted why reactivation occurs and why it happens differentially between the two classes of drugs tested. We make recommendations on how this issue can be combated.

Simulations predict that with an inoculum of 25 mycobacteria [32], latency is achieved (i.e., bacterial numbers are controlled) in fewer than 300 days, under appropriate immunologic conditions. Latent TB is characterized by low levels of extracellular bacterial load (<50 bacteria per cm$^3$ of granulomatous tissue), and all intracellular bacteria (Figure 1A) reside within a small number of chronically infected macrophages (MIs) (approximately 15 MI, with 50 intracellular bacteria each). The total population of T cells in latency (CD4+ and CD8+ T cells combined, Figure 1B and 1C) is comparable with numbers found experimentally, with a ratio of CD4+/CD8+ T cells approximately one, consistent with experimental observations [33,34]. During latency, TNF levels (Figure 1E) are on the order of 0.12 pg/mL (limited...
inflammation) as levels of Mls and MAs (activated macrophages) are relatively low (and these are major TNF producers). This small amount of TNF is significant, as neutralizing this concentration of TNF drives the system into active TB (see the section “TNF depletion and anti-TNF treatments”). This indicates a critical role for even small amounts of TNF in maintaining latency. Predicted ranges for IFN-γ and IL-10 (Figure 1D) all correlate with studies measuring cytokine levels at the infection site [35–37]. Total macrophage numbers do not change significantly in the first year post-infection, and resident macrophages remain relatively constant, while numbers of MIs and MAs remain below 50 (Figure 1F).

Sources of TNF during Latency

The roles played by different cellular sources of TNF involved in protective immunity remain unclear. During latency we evaluate and compare production of TNF by macrophages and lymphocytes (Table S2). The model predicts that macrophages are the main producers of TNF during the early phase of infection, and that once latency is achieved lymphocytes and macrophages produce similar amounts of TNF. This supports the idea that macrophages are key in establishing latency via TNF production, while T cell–derived TNF is essential, but not sufficient, for protection against Mtb infection, as shown in experimental data in mice [38].

Active TB

As discussed in the Methods section, by choosing different sets of parameter values, the mathematical model can simulate active infection. Active TB is characterized by uncontrolled growth of intracellular and extracellular bacteria throughout the simulation (500 days), reaching a total bacterial load of $10^8$ per cm$^3$ of granulomatous tissue approximately at day 300 (Figure 2A). Resident macrophage numbers drive cytokine dynamics in the first 300 days. When resident macrophage populations begin to fall (because they all become infected), a switch in bacterial populations occurs: extracellular bacteria are continuously increasing due to MIs bursting while intracellular bacteria reach a saturation level (determined by the level of available macrophages). High bacterial load is coupled to very high levels of IFN-γ (Figure...
2D) and TNF (~1000 pg/ml, see Figure 2E). Total macrophage population increases within the first 200 days, and by one year post-infection most of them are infected (see Figure 2F). T cell numbers (Figure 2B and 2C) are comparable with macrophage numbers during active TB (approximately 10^5 cells per cm^3 of granulomatous tissue). IL-12, IL-10 (Figure 2D), and IL-4 levels (Figure 2E) are also qualitatively and quantitatively similar to those observed in murine and NHP models as well as from limited human studies. For more details, see [31].

Uncertainty and Sensitivity Analysis

We investigate the importance of specific TNF-dependent mechanisms that allow for infection control via sensitivity and uncertainty analyses (see Methods). We observe how variations in different sets of parameters affect bacterial load. Table 2 illustrates TNF-dependent factors that, when varied, promote either lower bacterial levels (from latency) or increased bacterial load and reactivation of latent TB.

**TNF production.** Our sensitivity analysis indicates a critical role for TNF production by both MIs (x_{50}) and Th1 cells (x_{52}) throughout the infection (negative correlation values −0.6 to −0.2, p < 0.001, Table 2). TNF production by CD8+ T cells (x_{33}) is only significant in the first 250 days post-infection, suggesting that it is important for establishing latency but not maintaining it (see Table S2 for TNF production per cell type during latency).

**Cell Recruitment and Activation**

The model predicts that enhanced recruitment of lymphocytes (Th1, T8, and TC) is a desirable strategy toward establishing latency, as suggested by the strong and very significant negative correlation of TNF-dependent recruitment parameters (x_{3a} and x_{3b}) with bacterial load throughout the course of infection. On the other hand, macrophage activation plays a more important role in maintaining latency: the correlation between macrophage activation rate (k3) and bacterial load is significant only after latency is achieved (<1 year). TmTNF effects on macrophage (f_3) and lymphocyte activation (d) are not significant in either achieving or maintaining latency.

Among all TNF-related mechanisms, the uncertainty and
sTNF is almost completely deleted (where sTNF is the bioactive soluble form. We test variations on levels of so that the majority of tmTNF is cleaved and released in its transient expression of tmTNF in vitro [41], we assume that tmTNF $\equiv a + (1 - a)F_0$ represents tmTNF (where sTNF + tmTNF $= aF_0 + (1 - a)F_0 = (1 - a)F_0$. Considering the relative transient expression of tmTNF in vitro [41], we assume that $a$ is approximately 95% (i.e., only 5% TNF is transmembrane) so that the majority of tmTNF is cleaved and released in its bioactive soluble form. We test variations on levels of $a$ (percent sTNF) and report results in two settings in Figure 3. First we explore different percentages of sTNF ($a$) and look at the effects on bacteria load, and then we deplete different levels of $a$ after latency has been attained. Figure 3A shows bacterial load for different percentages ($a$) that is cleaved into sTNF in vivo. We introduce a parameter $a$ to indicate the fraction of TNF cleaved. The $a$ represents tmTNF (where sTNF + tmTNF $= aF_0 + (1 - a)F_0 = (1 - a)F_0$. Considering the relative transient expression of tmTNF in vitro [41], we assume that $a$ is approximately 95% (i.e., only 5% TNF is transmembrane) so that the majority of tmTNF is cleaved and released in its bioactive soluble form. We test variations on levels of $a$ (percent sTNF) and report results in two settings in Figure 3. First we explore different percentages of sTNF ($a$) and look at the effects on bacteria load, and then we deplete different levels of $a$ after latency has been attained. Figure 3A shows bacterial load for different percentages $a$ cleaved TNF. The system gradually shifts to higher bacterial loads with decreasing amounts of sTNF. This transition arises through oscillations that push the system to active TB when sTNF is almost completely deleted (where sTNF is $<5\%$ of total TNF). We obtain a similar dynamic during a depletion experiment where at day 500 (after latency is attained) we deplete varying levels of sTNF from the system (Figure 3B). The system reactivates when almost no sTNF is released. This suggests sTNF is necessary to control active TB and to maintain latency, likely because of its crucial role in lymphocyte and macrophage recruitment, and that tmTNF is not sufficient to maintain latency in humans, as seen in mice [42,43]. Figure S3 numerically shows how the stability of the latency state is dependent on $a$ and partially explains why sTNF is necessary to maintain latency (as shown in Figure 3B).

### Anti-TNF Therapies

We use the mathematical model to simulate three virtual clinical trials (VCT) of anti-TNF treatments (protocols are described in detail in Tables S3 and S4). The first two VCT are designed to explore which factors contribute most to reactivation of latent TB during two types of anti-TNF treatment. The third VCT explores the effects of exposure to Mtb after anti-TNF treatment is initiated.

Two classes of biological inhibitors were tested in the VCT: anti-TNF antibody and TNF receptor fusion (TNF2Fc). We define each drug as having a specific ability to neutralize TNF at the granuloma site; these data are not currently known (i.e., the drug neutralizing power). We define $TNF \text{ bioavailability}$ as the amount of total TNF available for use in the granuloma during anti-TNF treatment. Since we model TNF concentrations in granulomatous tissues, high bioavailability of TNF during therapy translates into a low neutralizing power of the drug or low penetration of the drug into granulomatous tissue.

As shown above (Figure 1), our simulation of the latent TB state predicts TNF levels at 0.12 pg/mL. This is the same order

### A Crucial Role of sTNF in Achieving and Maintaining Latency

Little or no data are available to indicate the fraction of TNF ($F_0$) that is cleaved into sTNF in vivo. We introduce a parameter $a$ to indicate the fraction of TNF cleaved. The $a$ represents tmTNF (where sTNF + tmTNF $= aF_0 + (1 - a)F_0 = (1 - a)F_0$. Considering the relative transient expression of tmTNF in vitro [41], we assume that $a$ is approximately 95% (i.e., only 5% TNF is transmembrane) so that the majority of tmTNF is cleaved and released in its bioactive soluble form. We test variations on levels of $a$ (percent sTNF) and report results in two settings in Figure 3. First we explore different percentages of sTNF ($a$) and look at the effects on bacteria load, and then we deplete different levels of $a$ after latency has been attained. Figure 3A shows bacterial load for different percentages $a$ cleaved TNF. The system gradually shifts to higher bacterial loads with decreasing amounts of sTNF. This transition arises through oscillations that push the system to active TB when sTNF is almost completely deleted (where sTNF is $<5\%$ of total TNF). We obtain a similar dynamic during a depletion

### Table 2. Uncertainty and Sensitivity Analyses of the Model for TNF-Related Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Correlation with Total Bacterial Load</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF production</td>
<td>$a_{30}$ Rate of production by MIs</td>
<td>Negative ($-0.5$ to $-0.3$)</td>
<td>Always very significant $^a$</td>
</tr>
<tr>
<td></td>
<td>$a_{31}$ Max rate of TNF production by MA (induced by either IFN or BT)</td>
<td>Positive</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$s_{10}$ Half-sat, IFN, and BT on TNF production by MA</td>
<td>Negative</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$s_{32}$ Rate of production by Th1 cells</td>
<td>Negative ($-0.6$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$a_{33}$ TNF production by TB</td>
<td>Negative ($-0.2$, $-0.1$)</td>
<td>Significant in the first 250 days</td>
</tr>
<tr>
<td>Macrophage activation</td>
<td>$k_3$ Activation rate of macrophages</td>
<td>Negative ($-0.15$)</td>
<td>Significant after 300 days</td>
</tr>
<tr>
<td>c8</td>
<td>Half-saturation of second stimulus for macrophage activation (by bacteria or TNF)</td>
<td>Positive ($0.1$, $0.2$)</td>
<td>Significant after 200 days</td>
</tr>
<tr>
<td>TNF-dependent recruitment</td>
<td>$k_{14a}$ Rate of TNF-independent recruitment of lymphocytes</td>
<td>Negative ($-$1)</td>
<td>Significant in the first week and between 120 and 160 days</td>
</tr>
<tr>
<td></td>
<td>$a_{3a}$ (TNF-independent recruitment of Th1)</td>
<td>Negative ($-0.7$, $-0.9$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$k_{14b}$ Rate of TNF-dependent recruitment of Th1</td>
<td>Negative ($-0.8$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$a_{3a}$ (TNF-dependent recruitment of Th8 and Th10)</td>
<td>Negative ($-0.7$, $-0.9$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$a_{3a}$ (TNF-dependent recruitment of Th8 and TC)</td>
<td>Negative ($-0.8$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$a_{3a}$ (TNF-dependent recruitment of Th8 and TC)</td>
<td>Negative ($-0.8$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td>Apoptosis (M1)</td>
<td>$\sigma$ Proportion of sTNF and tmTNF</td>
<td>Negative ($-0.9$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td></td>
<td>$k_{14a}$ Rate of CD4-dependent apoptosis</td>
<td>Negative ($-0.6$ to $-0.4$)</td>
<td>Always very significant</td>
</tr>
<tr>
<td>Reverse signaling (RS)</td>
<td>$f_3$ Direct “downregulation” effect of macrophage activation through tmTNF-reverse signaling</td>
<td>Negative</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>$d$ Extra T cell activation induced by RS</td>
<td>Positive ($0.3$)</td>
<td>NS</td>
</tr>
</tbody>
</table>

$^a$NS, not significant; significant ($p < 0.05$); very significant, $p < 0.001$.

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as those obtained via our granuloma homogenate data of 0.5 pg/ml derived from a nonhuman primate model (see Text S2).
When performing deletion and depletion experiments, we determined that only a small percentage of the total latency TNF was required to maintain latency (see Methods). At levels of TNF below that minimum, the system always reactivated. We assume treatment affects TNF bioavailability such that only a percentage of the total latency TNF is bioavailable. We define reactivation threshold (RT) as a level (percent of TNF in latency) of bioavailable TNF below which results in reactivation (see Methods). Thus, during anti-TNF treatment we can predict whether the bioavailable TNF is pushed below this threshold, leading to reactivation. If the RT is high, then more bioavailable TNF is required to maintain latency; if RT is low, only very low levels of bioavailable TNF are needed to maintain latency.

Virtual Clinical Trial 1

A series of VCT were simulated assuming different TNF bioavailability ranges induced by the two different treatments and a natural biological variation of \( \sigma \) (percentage of total TNF cleaved and released as sTNF). Considering the transient expression of tmTNF in vitro [41], we assume \( \sigma \) varies between 50% and 100%. Table 3 illustrates the results in

![Figure 3. Comparing the Roles of sTNF and tmTNF](A) Mathematical model simulations of total bacterial load corresponding to different proportions \( \sigma \) (percent sTNF versus tmTNF); all the other parameters are fixed to parameters yielding a latent state (see Table S6). (B) Simulated depletion of variable levels of sTNF. Until day 500, the system is in latency and \( \sigma = 0.95 \). Then at day 500, the depletion of sTNF is performed. Different values of \( \sigma \) are shown, where \( \sigma \) is the percent cleaved TNF (sTNF). Total bacterial loads are shown corresponding to different percentages of sTNF after day 500.
doi:10.1371/journal.pcbi.0030194.g003

<table>
<thead>
<tr>
<th>( \sigma ): Percent of Total TNF Cleaved</th>
<th>TNF Bioavailability during Treatment: Percent Left of Latency Level</th>
<th>Reactivation per 100 Patients Given Anti-TNF Antibody</th>
<th>Reactivations per 100 Patients Given TNF Receptor Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%–100%</td>
<td>&lt;20%</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>20%–30%</td>
<td>100</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>30%–50%</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Number of reactivations per 100 virtual patients using both TNF receptor fusion and anti-TNF antibody treatments.
doi:10.1371/journal.pcbi.0030194.s003
In addition to bioavailability, the percentage of total TNF cleaved (σ) may also be an important variable. First, we will assume that both molecules equally affect TNF bioavailability so we can explore independently the effects of sTNF versus tmTNF levels. Table 4 shows results for evaluating RT for different σ to investigate the role of tmTNF in TB reactivation during anti-TNF therapy. If we assume that σ is not very variable in the population and approximately equal to 95% (the baseline value that we used to generate our latency results), and we vary TNF bioavailability in the same range for both treatments (between 20% and 50%), our model predictions show that the differential reactivation risk is 34 per 100 anti-TNF antibody-treated virtual patients versus 28 per 100 TNFR2Fc-treated virtual patients (Table 4). RTs are not significantly different for this first experiment (25.11% versus 24.19%). However, if σ is allowed to vary from 50% up to 100%, as in VCT1, the VCT now predicts a differential reactivation risk of 46 per 100 anti-TNF antibody-treated virtual patients versus 30 per 100 TNFR2Fc-treated virtual patients (Table 4). RTs are now significantly different (28.62% versus 24.19%, p < 0.001). So, a lower σ (more tmTNF and less sTNF) has a negative impact on maintaining latency during anti-TNF antibody treatment.

To determine other factors that contribute to reactivation differences between the two therapies, we now fix both TNF bioavailability and σ. Since the RTs were not significantly different between the two treatments when σ is fixed at 95%, we isolate the effect of TNF bioavailability by fixing it at 28%. We chose this value of bioavailability specifically because it allows us to determine other factors responsible for more reactivation in the anti-TNF antibody therapy when no reactivations occur for the TNFR2Fc therapy (Table 4).

Our sensitivity analysis (Table S5) demonstrates that by varying all 12 parameters in the model that are affected by anti-TNF antibody treatment, only three contribute significantly to the observed reactivation. Cell loss rates of IFN-γ producing CD8+ cell (T8 [31]) and MA induced by anti-TNF antibody therapy positively correlated with total bacterial load (μMtTNF-MA and μMtTNF-MA).

The cell loss rate of MI negatively correlated with total bacterial load (μMtTNF-MI). Thus, anti-TNF treatment reduces the number of MAs and T8 cells and increases bacterial levels, increasing risk of reactivation. Although anti-TNF antibody also reduces the number of MIs, this is not sufficient to maintain latency. This may explain why a higher percentage of tmTNF has a negative impact on infection containment during anti-TNF antibody treatment: with more tmTNF, more MAs and T8 cells are lost from the granuloma.

Duration of treatment also affects risk of reactivation for both therapies. Table 5 shows simulation results where we varied simultaneously TNF bioavailability (between 20% and 50%) and σ (between 50% and 100%), setting different protocols for treatment duration. There is a significant difference in average RTs between a 12-month regimen and 18- or 24-month regimen for both treatments. We tested whether bacterial load at treatment initiation affects reactivation risk. If the total bacterial load is <500 CFU, no reactivation is observed for both treatments. If total bacterial load is 2–3 fold higher than latency level (~3–4e3 CFU), the system always reactivates for both treatments (unpublished data).

Virtual Clinical Trial 3

If treatment starts before infection with Mtb occurs, we assume that drug penetration is not relevant because the granuloma has not yet formed. We assume instead that a certain concentration of anti-TNF molecules are present in the lung where granulomas would begin to form in response to infection. Average serum concentrations of anti-TNF molecules are published [44] but no data are available for lung. It takes 300 days to achieve latency with TNF at 0.12 pg/ml.

Table 4. Virtual Clinical Trial 2 (VCT2)

<table>
<thead>
<tr>
<th>TNF Bioavailability during Treatment: Percent Left from Latency Level</th>
<th>Anti-TNF Antibody</th>
<th>TNF Receptor Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ (Percent sTNF)</td>
<td>Reactivations per 100 Virtual Patients</td>
<td>Reactivation Threshold</td>
</tr>
<tr>
<td>20%–50% 95%</td>
<td>34 25.11%</td>
<td>28 24.19%</td>
</tr>
<tr>
<td>50%–100% 46</td>
<td>28.62%</td>
<td>30 25.01%</td>
</tr>
<tr>
<td>29% 95%</td>
<td>95</td>
<td>—</td>
</tr>
</tbody>
</table>

*Very significant (p < 0.001)

Number of reactivations and RTs per 100 virtual patients using both TNF receptor fusion and anti-TNF antibody treatments. The reactivation threshold (RT) is defined in the Methods section. It represents a threshold where reduction of bioavailable TNF below that percent of latency value leads to reactivation.

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mL (see Figure 1). We capture changes in TNF bioavailability during anti-TNF therapy by dynamically lowering TNF levels by a constant percentage throughout treatment (Figure S1).

For level of total TNF 50% lower than baseline latency levels, treatment with either drug leads to disease, following acute infection, with no significant differences between them (Figure S2).

### Discussion

The major findings from this study are that bioavailability of TNF following anti-TNF therapy is the primary factor for causing reactivation of latent infection, that anti-TNF therapy will likely lead to numerous incidents of primary TB if used in areas where exposure is likely, and that sTNF—even at very low levels—is essential for control of infection.

Our model predictions (see Figure 3) recapitulate recent murine studies that tmTNF is sufficient to provide acute but not long-term control of Mtb infection [42,43]. We predict that ≥2% of total TNF needs to be released in soluble form to control acute infection and maintain latency. Bacterial loads increase as the percentage σ of total TNF cleaved is decreased, i.e., allowing more tmTNF in the system.

We use the model to analyze the effects of anti-TNF therapy by simulating anti-TNF antibody and TNFR2Fc. The reported measure unit for a steady state or average concentration of anti-TNF drugs in serum is in the order of pg/mL. Data on soluble TNFRs concentration in serum are on the order of ng/mL [45]. We use and predict concentrations of sTNF within the order of pg/mL based on our granuloma homogenate data (see Text S2). Given these predicted and reported concentrations, both treatments can systemically neutralize most if not all TNF. We can speculate that both TNFR2Fc and antibody will likely neutralize most if not all TNF at the granuloma site, if each penetrates granulomatous tissue equally well. An alternative way to interpret bioavailability is in terms of drug penetration into granulomatous tissues. However, our studies in murine models suggest that anti-TNF antibody penetrates or remains in the granuloma at higher levels than receptor fusion molecules [46]. The pharmacodynamic differences between these two agents include increased dissociation rate of TNF from TNFR2Fc compared with antibody [47]. Whether these differences play a role inside the granuloma is not known. However, one can imagine that increased dissociation in the context of high concentrations of endogenous TNF receptors could lead to better TNF signaling in the granuloma.

The VCT simulations suggest that TNF bioavailability is the main factor leading to reactivation by anti-TNF treatments in latently infected patients. Reactivation always occurs if both drugs penetrate the granuloma equally well (TNF bioavailability less than 20%). High bacterial load at treatment initiation increases the likelihood of reactivation. This suggests that a complete regimen of antibiotic treatment for Mtb infection prior to anti-TNF treatment could reduce the risk of reactivation. If TNF bioavailability is equally affected by the two treatments, differential cell level losses induced by anti-TNF antibody therapy accounts for higher reactivation rates: activated CD8+ T cells and MA loss are not compensated by the apparently beneficial effect of MI loss.

We speculate that the intracellular bacteria released after MI death induced by antibody binding to tmTNF (whether dependent on tmTNF reverse signaling or complement cascade) can only facilitate bacterial clearance by the host and does not enhance dissemination. Further, our results show that the longer patients are exposed to anti-TNF drugs through longer duration treatment protocols, the risk of reactivation increases. If infection with Mtb occurs after treatment is initiated, chances of developing active infection are very high if we assume reasonable levels of drug penetration into lungs (TNF bioavailability <50%). This is particularly important if anti-TNF treatments are implemented in regions of the world where infection risk is elevated. Bacteria grow uncontrolled when both sTNF and tmTNF are depleted (anti-TNF antibody therapy). These data suggest that tmTNF plays a key role in controlling active infection, where tmTNF preserves a subset of the beneficial mechanisms of TNF while lacking detrimental effects. Our predictions and recent experimental data [42] support the hypothesis that selective targeting of sTNF may offer several advantages over complete blockade of TNF in the treatment of chronic inflammatory diseases.

Current studies in both murine and NHP animal models by our group are now following up on these predictions. Our recent data from a mouse model showed that treatment with anti-TNF Ab in the chronic phase rapidly resulted in fulminant TB, while treatment with an etanercept-like molecule (receptor fusion) allowed mice to maintain control of the infection [46]. In contrast, following treatment with either antibody or receptor fusion during initial infection caused mice to succumb rapidly to the infection. This clearly

### Table 5. Duration of Treatment study for VCT2

<table>
<thead>
<tr>
<th>Treatment: Percent Left from Latency Level</th>
<th>σ (Percent sTNF)</th>
<th>Duration (Months)</th>
<th>Anti-TNF Antibody Reactivations per 100 Virtual Patients</th>
<th>Reactivation Threshold</th>
<th>TNF Receptor Fusion Reactivations per 100 Virtual Patients</th>
<th>Reactivation Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%–50%</td>
<td>50%–100%</td>
<td>12</td>
<td>46</td>
<td>28.62%</td>
<td>30</td>
<td>25.01%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>82</td>
<td>33.21%</td>
<td>67</td>
<td>30.34%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>85</td>
<td>33.2%</td>
<td>72</td>
<td>30.98%</td>
</tr>
</tbody>
</table>

Number of reactivations per 100 virtual patients using both TNF receptor fusion and anti-TNF antibody under conditions of VCT2. The reactivation threshold (RT) is defined in the Methods section.

*Very significant (p < 0.001).

doi:10.1371/journal.pcbi.0030194.s005
dM_{f} \over dt} = k_{2mR} \left( {B_{E} \over B_{E} + c_{0}} \right) - k_{17}M_{f} \left( {R_{2} \over R_{2} + (N_{2}M_{2})^{2}} \right) \\
- k_{14k}M_{f} \left( T_{c} + w_{1}T_{1} \over M_{1} \right) \\
- k_{31a}M_{f} \left( \sigma F_{x} + f_{id}I_{10} + s_{dI} \right) - k_{32}M_{f} \left( T_{c} + w_{1}T_{1} \over M_{1} \right) \\
- \mu_{M_{f}}M_{f} - \mu_{TNF-MI} \left( I - \sigma \right)F_{x} + s_{TNF-MI} \right) M_{f} \right) \\

\frac{dM_{a}}{dt} = k_{13}10R \left( I + f_{id}I_{1} + F_{1} \left( 1 - \sigma \right)F_{x} + s_{1} \right) \\
\times \left( B_{T} + \beta F_{x} \over B_{T} + \beta F_{x} + c_{0} \right) - k_{4}M_{a} \left( I_{10} \over I_{10} + s_{6} \right) \\
- \mu_{M_{a}}M_{a} - \mu_{TNF-MA} \left( I - \sigma \right)F_{x} + s_{TNF-MA} \right) M_{a} \right)

\text{Methods}

To better understand underlying dynamics of TNF production and function, we build on our mathematical model of Mtb in humans using 16 nonlinear ordinary differential equations. The updated model tracks three macrophage populations (resting, activated, and infected) and multiple T cell (Th0, Th1, Th2, and CD8+ T cell subsets) populations [31]. The model also includes five cytokine concentrations (IFN-γ, IL-12, total TNF, IL-10, and IL-4) and two bacterial (intracellular and extracellular) populations with numbers representing temporal dynamics of these populations in the lung (our modeling environment). The biological assumptions and the equations of the updated human Mtb model are outlined and described in the following section.

Model equations. The nonlinear ODE model is based on [31] and simulates interactions between two bacterial subpopulations, eight cell populations, and five cytokines. The new terms related to TNF dynamics are represented in bold and are described in the Methods section. Submodel diagrams are illustrated in Figures 4–6. They show macrophage, lymphocyte, and bacteria dynamics, capturing the terms represented for each of our equations. Cytokine production dynamics are superimposed on each diagram.

Macrophage dynamics. The equations describing dynamics for the macrophage subpopulations are given by:

\[
\frac{dM_{f}}{dt} = s_{Mf} + \mu_{M_{f}}(M_{f} + w_{M_{f}}) + s_{Mf} \left( \sigma F_{x} + f_{id}I_{10} + s_{dI} \right) \\
- k_{2}M_{f} \left( B_{E} \over B_{E} + c_{0} \right) - k_{17}M_{f} \left( R_{2} \over R_{2} + (N_{2}M_{2})^{2} \right) \\
- k_{14k}M_{f} \left( T_{c} + w_{1}T_{1} \over M_{1} \right) \\
- k_{31a}M_{f} \left( \sigma F_{x} + f_{id}I_{10} + s_{dI} \right) - k_{32}M_{f} \left( T_{c} + w_{1}T_{1} \over M_{1} \right) \\
- \mu_{M_{f}}M_{f} - \mu_{TNF-MI} \left( I - \sigma \right)F_{x} + s_{TNF-MI} \right) M_{f} \right) \\
\frac{dM_{a}}{dt} = k_{13}10R \left( I + f_{id}I_{1} + F_{1} \left( 1 - \sigma \right)F_{x} + s_{1} \right) \\
\times \left( B_{T} + \beta F_{x} \over B_{T} + \beta F_{x} + c_{0} \right) - k_{4}M_{a} \left( I_{10} \over I_{10} + s_{6} \right) \\
- \mu_{M_{a}}M_{a} - \mu_{TNF-MA} \left( I - \sigma \right)F_{x} + s_{TNF-MA} \right) M_{a} \right)
\]
carrying capacity of N, we assume that one-half of the MIs burst when the intracellular bacterial load reaches NMI. This mechanism has a maximal rate of \( k_{17} \), and is described by a Hill process. Immune responses also contribute to MI killing by several mechanisms. Both CD8+ and CD4+ T cells can use the Fas-FasL apoptotic pathway to induce apoptosis in these cells at a maximum rate \( k_{14} \). The half-saturation constant \( c_4 \) describes the effector-target ratio (T>MI) at which this process is half maximal. TNF can also induce apoptosis by binding to the TNFR1 receptor. This process is downregulated by IL-10 and occurs at a rate of \( k_{15} \). Finally, CTL killing by CD8+ and CD4+ T cells happens at a rate of \( k_{42} \). Specifically, CD4+ T cells have a limited contribution and this is accounted for by scaling the CD4+ T cell numbers (0 < \( w_1 < 1 \)). CD8+ T cell numbers are scaled by a Michaelis-Menten term accounting for the indirect dependence on CD4+ T cells for their killing capability. MAs are generated from their number (\( \mu_{MA} \)). MAs can be deactivated by IL-10 at a rate \( k_{4} \).

TB Reactivation in Anti-TNF Treatment

Figure 5 shows a descriptive diagram of CD4+ lymphocyte dynamics, with the terms captured for each of our equations. A similar diagram can capture the dynamics of CD8+ lymphocytes (Equations 7–9).

Similar to resting macrophages, recruitment of T cells occurs in both a TNF-independent and a TNF-dependent manner. The terms are similar, using different rates for the different T cell subsets (Equations 1–3, respectively). We assume that CD4+ T cells can arrive at the site of infection either as Th0 (majority), or a small fraction may arrive already differentiated into Th1 or Th2 cells (see Wigginton et al. [28] for a complete discussion).

Upon arriving at the site of infection, Th0 cells (Equation 4) can proliferate further in response to signals released by MAs at a rate \( \alpha_2 \).
Figure 6. Bacteria Dynamics
Descriptive diagram of bacteria dynamics implemented in the mathematical model in Equations 15–16.

**Cytokine Dynamics.**

\[
\frac{dF}{dt} = \alpha_{30} M_f + \alpha_{31} M_a (I_1 + I_2 + I_3 + I_4) + \gamma_0
\]  

\[
\frac{dI_1}{dt} = \frac{\beta_T}{B_T + \epsilon_1} I_2 + \alpha_{21} T + \gamma_0
\]  

\[
\frac{dI_2}{dt} = \frac{\beta_T}{B_T + \epsilon_2} I_2 + \alpha_{22} T + \gamma_0
\]  

\[
\frac{dI_3}{dt} = \frac{\beta_T}{B_T + \epsilon_3} I_2 + \alpha_{23} T + \gamma_0
\]  

\[
\frac{dI_4}{dt} = \frac{\beta_T}{B_T + \epsilon_4} I_2 + \alpha_{24} T + \gamma_0
\]  

**Bacterial dynamics.** Figure 6 shows a descriptive diagram of bacteria dynamics, with the terms captured for each of our equations.

Intracellular bacteria (Equation 15) grow at a maximal rate \(\gamma_0\) with logistic Hill kinetics accounting for a maximal carrying capacity of a macrophage. Extracellular bacteria (Equation 16) become intracellular when a macrophage becomes chronically infected at an assumed threshold of N2 bacteria, and hence this represents a gain term for the intracellular bacteria. Bursting of macrophages (k17) adds to the extracellular subpopulation. To account for loss of intracellular bacteria due to various killing mechanisms, we assume each killed MI to hold an “average” number of bacteria, given by \(N_{AVG} (<\infty)\). The corresponding gain in extracellular bacteria depends on the mechanism of killing: while Fas-FasL–induced apoptosis (k14a) releases all intracellular bacteria, TNF-induced apoptosis (k14b) eliminates approximately 50% of the bacteria within the macrophage, and this is shown by the \(N_{AVG}\) multiplier in the BE (extracellular bacteria) equation (Equation 16). CTL activity (k32) kills virtually all the intracellular bacteria, and does not add on to the BE (extracellular bacteria) population. Lastly, we assume that natural death of MIs also releases all intracellular bacteria, and this is modeled as a constant turnover of the bacteria (k40) from intracellular to extracellular. Extracellular bacteria grow at a maximum rate \(\gamma_0\). They are taken up and killed by activated and resting macrophages at rates \(k_{15}\) and \(k_{16}\), respectively.

Th0 cells can also differentiate into Th1 (Equation 5) and Th2 (Equation 6) cells. Th1 differentiation is controlled by IL-12 and IFN-\(\gamma\) and opposed by IL-4 and IL-10. Th2 differentiation is induced by IL-4 and opposed by IL-10. Th2 cells die at a rate \(\gamma_0\) and die at a rate \(\gamma_0\). Th1 cells can be killed due to IFN-\(\gamma\) induced apoptosis at a peak rate \(\gamma_0\). There are two Th1 and Th2 cells die naturally at rates \(\gamma_0\) and \(\gamma_0\), respectively. As is the case for CD4 cells, we assume that CD8+ T cells can arrive at the site of infection as T80 (Equation 8) or TC (Equation 9) type. T80 cells are activated due to interaction with Th1 and Th2 cells and cytokines and have a natural half-life. CD8+ T cells also undergo IFN-\(\gamma\) induced apoptosis at a peak rate \(\gamma_0\), and die at a rate \(\gamma_0\). Since the T8s (Equation 8) and TCs (Equation 9) are functional subsets of the CD8+ T cell population (see Introduction), the equations are identical for both. We introduce a parameter \(m\) that accounts for possible overlap between T8 and TC subsets. This assumption is further studied in the CD8+ T cell kinetics section of Sud et al. [31].
TB Reactivation in Anti-TNF Treatment

Complement cascade is the release of intracellular bacteria, while apoptosis kills most of the intracellular bacteria [53]. A direct “downregulation” effect of macrophage activation through tmTNF reverse signaling (macrophage anergy or LPS resistance) is included in the macrophage activation term \( k_9 \) as follows (bold term):

\[
k_9 M_{B_1} \left( \frac{F_{T1} + F_{NMI}(1-\sigma) \omega_1 + \omega_2}{1 - \sigma + \omega_2} \right) B_1 + \frac{F_{T1}}{B_1 + \omega_2 + \nu}
\]

We do not directly include LT \(_a\) in the model, but we indirectly account for LT \(_a\)-dependent recruitment of macrophages and lymphocytes during anti-TNF therapy (namely TNF receptor fusion protein), since TNF receptor fusion protein binds LT \(_a\) while anti-TNF antibody does not (see Table S3). We differentiate the two treatments by downregulating all TNF-independent recruitment terms during receptor fusion treatment simulations.

**Computer simulations.** Once we derive the model and estimate parameters, we solve the system of 16 nonlinear ordinary differential equations to obtain temporal dynamics for each variable. To this end, we used Matlab version 7.0.1.83 (R14) Service Pack 3 (The Mathworks) platform and its numerical methods together with a computer code using a different solver written by our group.

As discussed previously [28,29,31], we chose total bacterial load as a marker of disease, where bacterial levels can distinguish between two different scenarios: latent infection (steady state, low total bacterial levels) and active disease (unchecked bacterial growth).

**Parameter estimation.** Before simulations can be performed, parameters must be estimated from literature sources or by mathematical means. Values for most model parameters are estimated from published experimental data or data generated from our group. Data from human studies and Mtb experiments are favored over mice and other mycobacterial species, respectively. Where no appropriate data is available for a given parameter, we conduct uncertainty analysis to obtain a range within orders of magnitude. A detailed description of techniques used to evaluate model parameters, as well as a listing of parameters already estimated can be found in work previously published by our group [31]. All parameters newly estimated for the purpose of this work are listed in Table S6, together with parameters previously estimated. All parameters have been estimated using approaches similar to those described in Wigginton et al. [28].

Parameter values represent mechanisms in the host–pathogen system, and these were estimated from many different experimental sources. There is great variation that likely exists among them. In previous work [31], we explored wide ranges on these parameter values to determine how the system changes when values change. A group of parameters were identified as being key determinants between the host–pathogen system achieving latency or going to active disease (see Table I and II in [31]). These different infection states are obtained by varying parameter values, as discussed in the next section. The set of parameters that we used to generate model parameters, as well as a listing of parameters already estimated can be found in work previously published by our group [31]. All parameters newly estimated for the purpose of this work are listed in Table S6, together with parameters previously estimated. All parameters have been estimated using approaches similar to those described in Wigginton et al. [28].

Uncertainty and sensitivity analysis.** There is an intrinsic biological and experimental variability in rates measured from in vivo or in vitro studies. Further, some interactions in the Mtb-host system are not currently measurable, particularly at the level of the lung granuloma. This complicates accurate estimation of model parameters (baseline values are unknown).

We quantify the importance of each TNF-related mechanism involved directly and indirectly in the infection dynamics using both uncertainty and sensitivity analyses as described previously [31]. The statistical techniques of Latin hypercube sampling (LHS) and partial rank correlation (PRC) [54–57] when combined guide our understanding as to how and to what extent variability in parameter values affects infection outcomes. We employ the LHS method to control effects of uncertainties in our parameter estimation by varying all the TNF-related parameters simultaneously. LHS allows for simultaneous random, evenly distributed sampling of each parameter within a defined range (stratified Monte Carlo technique). The sampling is done by sampling each parameter over a wide range to produce a factor of 1,000 above and below reported literature data or mathematical estimates) and performing a large number of computer simulations \( n \) (is significantly large). The stochastic approach allows for a global sensitivity study as compared with a deterministic analysis that gives local sensitivity based on the sensitivity equation. The major drawback of the deterministic approach is that the results are based on baseline values (often unknown) for the parameters involved in the sensitivity equations. The LHS approach does not suffer from this limitation.
The PRC method allows us to correlate the variability observed using the LHS method and to determine which parameters are responsible for the variation in outcomes. PRC coefficients (PRCs) are between -1 and 1 and have a standard p-value that indicates significance. A negative PRC coefficient indicates that a decrease in the value of that parameter results in an increase of the bacterial load. A positive PRC coefficient indicates that a decrease in the value of that parameter results in a decrease of the bacterial load. PRC coefficients also evaluate temporal changes in the significance of these parameters as they relate to bacterial load at different times during the infection. Statistical significance of these correlations is assessed by a generalized t-test (see the “Statistical analysis” section). For example, the rate of TNF production by Th1 cells ($\sigma_{23}$) is always very significant and negative (see Table 2): if we lower TNF production by Th1 cells, bacterial load increases.

**Virtual deletion and depletion.** As a way to validate the mathematical model, we recapitulate experimental approaches such as TNF gene knockouts and TNF neutralization studies. These can be simulated with our mathematical model as virtual deletion and depletion simulations, respectively. Virtual deletions remove an element from the system at day zero while virtual deletions mimic experimental conditions where an element can be depleted or neutralized via antibody treatment at any time during the infection. We can selectively delete or deplete sTNF or transmembrane TNF (tmTNF) by varying the parameter $\sigma$ prior to infection (Figure 3A) or after latency is achieved (i.e., at day 500 post-infection, Figure 3B). Setting $\sigma$ to zero mimics sTNF deletion/depletion, while tmTNF deletion/depletion is obtained by setting $\sigma$ to 1. We restrict our results to virtual TNF deletion/depletion studies to investigate the role of TNF during active and latent TB. Previous deletion and depletion experiments were performed for all of the relevant cells and cytokines in the mathematical model (see [31] for details).

When all the TNF is deleted from the system on the same day that infection is initiated, the system goes to active disease (see Figure 7, TNF$^{-/-}$). This occurs with low-level cellularity, i.e., macrophage numbers are almost an order of magnitude lower (mainly infected and activated) than when disease occurs in the wild-type scenario (see Figure 2). This is consistent with studies that report diffuse infection, where disease is spread throughout a large lung area, resulting in an overall lower cellular density and widespread tissue damage [2–4,11]. Upon total TNF depletion (performed at day 500 post-infection), the system progresses to a disease state in fewer than 100 days (see Figure 7, TNF depl). Depletion reduces the total number of macrophages to 25% (unpublished data), consistent with recent studies [6]. T cells are depleted upon TNF removal mainly because they turn over and are not quickly replenished; they then recover due to compensatory recruitment by other TNF-independent mechanisms in response to high bacterial levels. Thus, the depletion simulations suggest that although TNF is present at extremely low levels during latency ($\sim 0.12$ pg/mL of granuloma homogenate, see Figure 1), this low level is necessary and sufficient for control and maintenance of infection. This finding is further confirmed later in the anti-TNF treatment simulations. Our results also indicate that control of infection is a dynamic, TNF-dependent process involving continual cell turnover, an outcome that is consistently observed across experimental studies [8].

**Simulated TNF blockade in tuberculosis infection: Anti-TNF antibody versus TNF receptor fusion.** The US Federal Drug Administration monitors the safety of TNF inhibitors through its Adverse Event Reporting System (AERS), a surveillance system to which drug manufacturers are required to submit reports of adverse events and to which health care professionals and consumers voluntarily send adverse event reports. Wallis et al. [27] published a systematic study of granulomatous infections associated with infliximab and etanercept contained in AERS, using reports from 1998 (when the two drugs were approved) through the third quarter of 2002. TB is the most frequent disease, reported in $\sim 144$ per 100,000 patients (infliximab-treated patients) and in $\sim 35$ per 100,000 patients (etanercept-treated patients). Although the clustering of adverse events reported shortly after initiation of infliximab treatment is consistent with reactivation of latent infection, the number of infected individuals with latent TB is not reported in the AERS database for both treatments. There is a possibility that some TB cases result from infection after therapy is initiated. Anti-TNF antibody treatment (such as with infliximab) targets both sTNF and tmTNF. We also account for additional cell loss due to tmTNF engagement by the drug TNF receptor fusion treatment (such as with etanercept) targets sTNF and L122. We capture the action of these two TNF neutralizing drugs by including an additional loss term in the TNF equation. This term accounts for concentration-dependent loss of TNF as a function of a drug’s half-life, dissociation rate, bioavailability, and treatment regimen. Table S3 shows data for pharmacokinetics (PK), pharmacodynamics (PD), and treatment protocols (doses and administration) for both drugs. Since our modeling approach describes average dynamics within a granulomatous tissue sample in the lung (see the section “Measure units and modeling space” for details), we define percentages of neutralized

![Figure 7. Simulations of Total TNF Deletion and Depletion](image-url)

**Figure 7.** Simulations of bacterial loads during TNF deletion (TNF$^{-/-}$) and depletion (TNF depl). The y-axis represents total bacterial load. Latency is our wild-type control (see Figure 1). Note, $\alpha = 0.95$ for these simulations. doi:10.1371/journal.pcbi.0030194.g007
sTNF and/or tmTNF (biow) that capture the overall neutralizing power of either receptor fusion or antibody in granulomatous tissue.

**Anti-TNF antibody.** Infliximab is a human–mouse chimeric monoclonal TNF antibody that binds potently and essentially irreversibly to monomeric and trimeric TNF, both soluble and membrane-bound, but does not bind to biologically inactive LTα [47]. Further, due to its interaction with tmTNF, infliximab induces apoptosis in TNF-producing cells (including Mbs and Mas, CD4+ and CD8+ T cells) via a caspase-dependent pathway [52]. Binding of infliximab to one subunit of trimeric TNF leaves additional subunits free to interact with other anti-TNF antibodies, raising the possibility of formation of immune complexes, under certain conditions (i.e., high TNF levels).

**TNF receptor fusion.** Another anti-TNF drug, etanercept, is a TNF receptor p75-lig fusion protein rather than an antibody. It binds selectively to human trimeric sTNF and LTα3, with a 4-fold lower affinity with respect to infliximab [19,47]. As a result, the effect of a receptor fusion drug is deleterious of sTNF, but is less potent in binding tmTNF; thus, little or no apoptosis has been observed in clinical data with this drug. The frequency of reactivation of TB in etanercept-administered patients appears to be lower than for infliximab [27], although head-to-head comparison of the two drugs in human studies have not been performed. While it is possible that differences in apoptotic activity of infliximab and etanercept yield these contrasting results, it is more likely that apoptosis coupled with concentration and timing of TNF neutralization leads to the different outcomes between the two drugs.

Receptor fusion has a fast dissociation rate: it sheds 50% of sTNF and 90% of tmTNF in only 10 minutes, but can bind TNF again immediately [47]. Thus it is possible that the TNF-neutralizing effect is intermittent: molecules of sTNF and LTα3 are engaged for very short periods of time, allowing for a redistribution of TNF throughout the system. However, if receptor fusion concentration is relatively high, those released molecules will quickly be reassociated with the receptor fusion and are therefore not free in the system. In contrast, under situations where TNF is released from the receptor fusion molecules and there are high numbers of cell-associated TNF receptors present (such as in a granuloma) and possibly a lower level of receptor fusion (due to poor penetration), TNF might bind to the cell-associated TNFR1 or TNFR2 instead of back onto the receptor fusion. This contributes even more to lowering levels of bioavailable TNF in granulomas during receptor fusion treatment.

We indirectly test LTα-neutralization in the model by lowering all the TNF-independent recruitment parameters using the bioavailability coefficient (bioav): we assume that LTα-neutralization is of the same magnitude as sTNF neutralization.

**Virtual clinical trials.** We perform three VCTs to investigate what factors contribute most to reactivation during anti-TNF treatments if patients are latently infected or if exposure/infection occurs after anti-TNF treatment is initiated.

Several factors and mechanisms hypothesized to be involved in TB reactivation by anti-TNF drugs can be tested. These include the differential power of the drugs to neutralize TNF bioavailability [47], differential inhibition of TNF signaling events (TNFR1/TNFR2 protein ratio expressed on cell surfaces) as a possible path for a cell to direct the consequences of TNF signaling [30]), and differential induction of target cell death induced by anti-TNF antibody binding to tmTNF [19,52]. Using the model, we can directly test the power of the drugs to neutralize TNF by varying the bioavailability parameter bioav. We can simultaneously explore differential cell level losses by varying tmTNF-related parameters. Since we model TNF receptors, we currently cannot address the other hypothesis (TNFR1/TNFR2 protein ratio). Finally, we investigate both the role of different bacterial loads during latency at treatment initiation and the duration of therapy as additional factors affecting risk of reactivation. To test whether bacterial levels play a role in reactivation rates, we vary two parameters that yield latency scenarios with higher bacterial levels before initiation of treatment (i.e., maximal rates of macrophage activation and CTL killing). The duration of both anti-TNF antibody and receptor fusion treatments varies between 12 and 24 months. We classify a virtual patient as undergoing TB reactivation when the bacterial load grows larger than 10^5 (latency level) during or after the end of the treatment. See Table S4 for details on the VCT settings.

**Reactivation threshold.** We define a reactivation threshold (RT) as a threshold where reduction of bioavailable TNF below this threshold level leads to reactivation. This value is expressed as a percentage of the TNF concentrations defined from the latency value. Each VCT comprises 100 simulations, where TNF bioavailability is varied in a specified interval. Each run is classified based on the bacterial load level, and reactivation is defined when bacterial loads grows uncontrolled. We define the reactivation subset of the 100 runs as the collection of all the reactivation cases with their bioavailabilities (from the uncertainty analysis). We obtain our RT as the average TNF bioavailability calculated on the reactivation subset. We statistically compare RTs between different trials by a standard t-test (see the “Statistical analysis” section).

**Measure units and modeling space.** Contradictory data exist regarding levels of sTNF and sTNF receptors in lung epithelial lining fluid obtained by bronchoalveolar lavage [45,59] in active pulmonary TB and healthy subjects. Very limited data are available on concentration profiles of TNF antagonists outside serum. We can assume that the concentration of the drug in the plasma (or serum) is proportional to the average drug concentration in its whole volume of distribution. The lung is highly vascularized, so average concentrations in plasma could be reasonable proxies for the average concentration of the drugs in the lung. This could be accomplished mathematically by finding a physiological value that translates blood to lung (i.e., volume to space) to account for bioavailability. However, this may not adequately represent diffusion of drug from blood vessels into consolidated granuloma tissue/caseum.

Average steady-state concentrations of anti-TNF antibodies [60] and TNF receptor fusion molecules [61] in serum are a function of the protocol (dose and administration) and type of pathology (see Table S3). TNF receptor fusion ranges from approximately 1 µg/ml up to 6 µg/ml. Anti-TNF antibody ranges from 8 µg/ml to 60 µg/ml (see [44] for details). The modeling space of our most recent model [31] is the whole human lung. Here we adapted that model to represent cellular and bacterial dynamics as number of cells or bacteria per cm^3 of granulomatous tissue and we describe cytokine concentrations in pg/mL of granuloma homogenate.

**Statistical analysis.** We perform PRC (partial Spearman correlation on rank-transformed data) and t-test (one-tail, two-sample unequal variances) with Matlab. See the Uncertainty and Sensitivity Analyses section for more details.

**Supporting Information**

**Figure S1.** Levels of TNF during Virtual Clinical Trial 3 during Both Anti-TNF Treatments

Found at doi:10.1371/journal.pcbi.0030194.sg001 (152 KB PDF).

**Figure S2.** Total Bacterial Loads during VCT3 for TNF Receptor Fusion (A) and Anti-TNF Antibody (B) Treatments

Found at doi:10.1371/journal.pcbi.0030194.sg002 (130 KB PDF).

**Figure S3.** Plot of the Gradient versus σ (Percentage of Soluble versus Transmembrane TNF)

The x-axis represents the 16 variables of the ODE system (1–16). The y-axis represents the 16 variables of the ODE system (1)- (16). The y- axis represents |y| / |max(y)|, σ on a log scale.

Found at doi:10.1371/journal.pcbi.0030194.sg003 (54 KB PDF).

**Table S1.** Summary of Effects of TNF and TNFR1 Blocking during Experimental M. tuberculosis Infection in Mice

Found at doi:10.1371/journal.pcbi.003194.sf001 (27 KB DOC).

**Table S2.** Model Simulations of Total TNF Production per Macrophages and T Cell Sources for Different Times

Found at doi:10.1371/journal.pcbi.0030194.sf002 (20 KB DOC).

**Table S3.** Anti-TNF Antibody and TNF Receptor Fusion, Pharmacokinetics, Pharmacodynamics, and Treatment Protocols

Found at doi:10.1371/journal.pcbi.0030194.sf003 (21 KB DOC).

**Table S4.** Summary of Virtual Clinical Trial Protocols and Simulation Conditions

Found at doi:10.1371/journal.pcbi.0030194.sf004 (22 KB DOC).

**Table S5.** Sensitivity Analysis for Anti-TNF Antibody Treatment Simulations

Found at doi:10.1371/journal.pcbi.0030194.sf005 (21 KB DOC).

**Table S6.** Parameter Table

New Parameter Estimates in addition to those estimated previously [28,31] (shown in parentheses are the values used to generate a latent state, see Figure 1).
References


