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MODELLING THE HUMAN IMMUNE RESPONSE MECHANISMS TO MYCOBACTERIUM TUBERCULOSIS INFECTION IN THE LUNGS

Gesham Magombedze

Department of Applied Mathematics
National University of Science and Technology
PO Box AC939 Ascot, Bulawayo, Zimbabwe

Winston Garira

Department of Applied Mathematics
National University of Science and Technology
PO Box AC939 Ascot, Bulawayo, Zimbabwe

Eddie Mwenje

Department of Applied Biololgy
National University of Science and Technology
PO Box AC939 Ascot, Bulawayo, Zimbabwe

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Abstract. This work elaborates on the effects of cytotoxic lymphocytes (CTLs) and other immune mechanisms in determining whether a TB-infected individual will develop active or latent TB. It answers one intriguing question: why do individuals infected with *Mycobacterium tuberculosis* (Mtb) experience different clinical outcomes? In addressing this question, we have developed a model that captures the effects of CTLs and the combined effects of CD4+ helper T cells (Th1 and Th2) immune response mechanisms to TB infection. The occurrence of active or latent infection is shown to depend on a number of factors that include effector function and levels of CTLs. We use the model to predict disease progression scenarios, including primary, latency or clearance. Model analysis shows that occurrence of active disease is much attributed to the Mtb pathogen ability to persist outside the intracellular environment and that high levels of CTLs result in latent TB, while low levels of CTLs result in active TB. This is attributed to the CTLs' ability to directly kill infected macrophages and the bacteria inside the infected macrophages. The study suggests directions for further basic studies and potential new treatment strategies.

1. **Introduction.** Tuberculosis (TB) remains the single largest infectious disease causing high mortality in humans. The majority of cases occur in developing countries, with sub-Saharan Africa having the highest incidence rate per capita [3]. In South Africa alone, two million people coinfected with *Mycobacterium tuberculosis* (Mtb) and human immunodeficiency virus (HIV) [3] were reported by the year 2000. Today Mtb remains one of the most ubiquitous pathogens in the world. Estimates are that one third of the world's population is infected with the bacillus and that it

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is responsible for 8 to 12 million cases of active TB and 3 million deaths each year [26]. Trends indicate that TB is on the increase and if proper measures are not taken to educate people about this preventable disease, infection rates will continue to rise at an alarming rate. TB strikes people of all races, ages and income levels. However, certain groups are at a higher risk, that is, people with close contacts with infectious TB, the poor, medically under-served people, homeless people, people in jail and immuno-compromised people.

Mtb is a slowly growing, facultative intracellular pathogen that can survive and multiply inside macrophages and other mammalian cells. The bacilli spread from the site of initial infection in the lungs through the lymphatics or blood to other parts of the body. The apex of the lungs and the regional lymph nodes are favored sites. After entry of the bacilli, phagocytosis of Mtb by alveolar macrophages takes place. Cell-mediated immune response develops within 2 to 6 weeks, and this is followed by an influx of lymphocytes and activated macrophages into the lesion, resulting in granuloma formation. The bacilli are then contained in the granuloma, where they can remain forever or be re-activated later or discharged into the airways, after increasing enormously in number. After infecting the lungs, the Mtb pathogen multiplies in the alveolar macrophages. The bacteria prefers intracellular growth as opposed to extracellular growth. Following infection a small percentage of people progress to primary TB within 1-2 years [18]. In most cases control of the infection occurs, leading to a clinically latent infection [18]. This latent infection can be maintained for the lifetime of the host with no clinical symptoms and no obvious adverse effects. The reactivation of the latent infection occurs in 5-10% of infected persons, and can be triggered by immunosuppression due to age, malnutrition, infection with HIV, or other factors [18]. Such people have a much higher risk of developing active TB following infection.

The immune response following the first exposure to Mtb is multifaceted and complex. It usually develops in the alveoli of the lungs, and alveolar macrophages are an ideal target for Mtb. When droplets containing Mtb are inhaled, the bacteria are ingested by resident alveolar macrophages and begin to multiply [2]. The bacteria multiply inside the macrophages up to a threshold limit, after which the macrophages burst, releasing more bacteria. In their resting state, not only are alveolar macrophages poor at destroying the bacteria, but Mtb can also inhibit their ability to kill phagocytized bacteria, most likely by preventing phagosome-lysosome fusion [7, 4, 5]. Clearance of resident bacteria by alveolar macrophages depends on the presence of lymphocytes as well as activation by interferon-gamma ($IFN-\gamma$), released by type 1 T cells (Th1) and other cells of the immune response that migrate to the site of infection in response to chemotactic signals generated by infected macrophages [6]. If the macrophage fails to receive sufficient stimulation for activation, it is unable to clear its resident bacteria. These chronically infected macrophages eventually either die due to a large number of resident bacteria or are destroyed by a CTL response. Bacteria are either killed when their host cell is destroyed or released, becoming, at least temporarily, extracellular. These bacteria may either infect resting macrophages or be ingested and killed by activated macrophages. After encountering the Mtb bacteria, resting macrophages release cytokines, which trigger activation of the macrophages. The extracellular bacteria can also encounter immature dendritic cells (IDC), which engulf them and migrate

to a draining lymph node (DLN) for antigen presentation. This prompts the mounting of an adaptive cell-mediated immune response. The dendritic cells also mature, becoming more efficient in antigen presentation.

Immunologic control of Mtb infection is based on a Th1 response. IL-12 is induced following phagocytosis of Mtb, which drives development of Th1 response [11, 15]. Mtb are strong IL-12 inducers, and so Mtb infection can skew the response to a secondary antigen toward a Th1 phenotype [18]. IFN-γ is another central cytokine in control of Mtb. This cytokine is produced both by CD4+ T cells and by CD8+ T cells and is important in macrophage activation [18, 8, 19]. T cells are responsible for killing infected macrophages that are unable to destroy their resident bacteria. This is accomplished through a Fas-Fas ligand apoptotic pathway by CD4+ T cells [23, 11, 24] and through other cytotoxic mechanisms, such as granule and perforins produced by CD8+ T cells and possibly CD4+ T cells [18, 10, 9, 8]. CD4+ T cells are involved in recognition of antigens that have been processed in phagosomes and presented as small peptide fragments in the context of MHC class II molecules on the surface of antigen-presenting cells such as monocytes, macrophages or dendritic cells. CD8+ T cells, on the other hand, recognize antigens that have been processed in the cytosol and that are presented in the context of MHC class I molecules on the cell surface. In general CD4+ T cells help to amplify the host immune response by activating effector cells and recruiting additional immune cells to the site of disease, whereas CD8+ T cells are more likely to be directly cytotoxic to target cells. Serbina et al. [25] examined the effect of CD4+ T cells' deficiency on the development of cytokine production and cytotoxic function of CD8+ T cells during acute tuberculosis. Serbina's work [25] indicated that priming and amplification of mycobacteria-specific CD8+ T cells occur.

There are theories surrounding the pathogenesis of TB in patients that try to explain why individuals have different disease outcomes after exposure to Mtb. Several of these immune theories focus on the central role of Th1/Th2 cross-regulation. It has been hypothesized that there is a switch from Th1- to a Th2-dominant cell-mediated immune response, leading to active disease. However, attempts at isolating Th2 cells and Th2-type cytokines from the site of infection have not always been successful [17, 16]. A second hypothesis is that a true switch from Th1 to Th2 response does not necessarily occur but instead the relative strength of the Th1 response determines latency or active disease [22, 19]. A paradigm was initially developed in a murine model on the function of CD4+ T cells and manifestations of disease, which has now accumulated a substantial amount of support in a variety of human diseases. The paradigm holds that CD4+ helper T cells can be separated into at least two phenotypic classes, Th1 and Th2 [26]. These cells are derived from Th0 or null cells and differentiate from the precursor cells under control of cytokines such as interleukin-12 (IL-12) [11]. Phenotypically, Th1 cells are characterized mainly by their ability to produce the cytokines IFN-γ and IL-12, whereas Th2 cells produce cytokines such as IL-4, 5 and 10.

A lung model was previously developed by Wigginton and Kirschner [11]. The model describes what happens at the site of infection, emphasizing the macrophage and lymphocyte interactions. Marino and Kirschner [12] extended the model to a two-compartmental model, which captures the interactions of the immune cells and Mtb in the lungs and in the lymph nodes. The motivation for this work is to improve the work done by Wigginton and Kirschner [11]. Their work did not consider the effects of granulysin and CTLs that enable infected macrophages to

clear their bacterial load. It has been shown that the toxic effects of granulysin and the cytotoxic effects of CTLs lead directly to the destruction of intracellular bacteria [13, 14]. In their work 2001 [11], Wigginton and Kirshner did not consider the effects of cytotoxic T cells separately, that is, lysis of chronically infected macrophages by CTLs and the possible killing of extracellular bacteria by exocytosis effects of CTLs and intracellular direct killing by antimicrobial toxic effects of granulysin, which has been shown to directly kill intracellular and extracellular bacteria [13, 14]. In their study [11], they captured the dynamics of cytokines, which are secreted as a result of antigen recognition by infected macrophages, as well as those secreted by activated macrophages, CD4+ T cells and CD8+ T cells. They managed to explain and predict possible disease outcomes due to the dynamics of these cytokines. In this work we do not capture directly the cytokine effects but consider their effects through the cells which secrete these cytokines (IL-4, IL-5, IL-10, IL-12 and IFN-γ). Also in their model [11], Wigginton and Kirschner made the point that Th2-type immune response results in active disease and that Th1 immune response results in either active or latent disease or control of the disease. This work will not consider the Th1/Th2 immune types separately but will consider the CD4+ helper T cell population as the sum of Th1 type and Th2 type. The main thrust and motivation for this work is therefore to investigate the effects of CTLs in immune response to Mtb infection.

2. **Model development.**

We develop a model for human TB at the site of infection in the lungs. The model outlines interactions among two bacterial populations, three macrophage populations, CD4+ T cells and CD8+ T cells.

2.1. **Macrophages.**

Resting macrophages are normally present in the lungs [12]. We assume that macrophages have a natural constant turnover, which is a source of new cells coming into the lungs due to monocyte differentiation. During infection, resident macrophages (M_R) undergo three different dynamics: (a) enhanced recruitment, (b) infection (M_I), and (c) activation (M_A). Recruitment is triggered with a chemokine gradient by bacterial density at the site of infection, which also contributes to infection of resting macrophages and their activation. Chronically infected macrophages die by bursting as a result of failing to clear their bacterial burden, or they can also die from CD4+ T cell-induced apoptosis or as a result of lytic killing by CTLs.

2.2. **Bacteria.**

We model two bacterial populations in the model: (a) intracellular bacteria (T_{BI}), which are found inside the macrophages as a result of Mtb uptake by the macrophages, and (b) extracellular bacteria (T_{BE}). The two bacterial populations multiply. Their location (intracellular and extracellular) dictates growth rates and the way they solicit the immune response. Extracellular bacteria are killed by both activated and resident or resting macrophages, while intracellular bacteria are killed by both resting and activated macrophages, as well as by the toxic effects of granulysin produced by CTLs. The total of intracellular and extracellular bacteria, (T_B) is given by $T_B = T_{BI} + T_{BE}$. We assume that macrophages which fail to clear their bacterial load become chronically infected and will eventually burst, releasing N bacterial particles [11]. Extracellular bacteria become intracellular when their host macrophage becomes chronically infected, and such a macrophage carries approximately one-half of its maximal carrying capacity ($\frac{N}{2}$) [12].

2.3. **CD4+ T lymphocyte cells.** T cells arrive at the site of infection as fully differentiated Th1 or Th2 cells or as Th0. Their recruitment is induced in response to signals released by infected and activated macrophages. Th0 may further differentiate into Th1 or Th2 cells, which is a result of cytokine effects. Since effects of cytokines are not being captured directly by our model, the population of CD4+ helper T cells is given by $T = Th1 + Th2$. We assume that induced apoptotic death of chronically infected macrophages result in the release of N_T bacterial particles from the intracellular compartment where $N_T < N$.

2.4. **CD8+ T cytotoxic cells.** CD8+ T cells are supplied from the thymus and they are further recruited to the site of infection in response to chemotactic signals generated by infected macrophages [6]. CTLs have been shown to kill both extracellular and intracellular bacteria and also can lyse chronically infected cells [13, 14]. It has also been observed that when CD4+ T cells count decrease in HIV-1 infected persons, the risk of tuberculosis is increased either from primary infection or from reactivation of latent Mtb infection [25]. We assume that chronically infected macrophages release N_C bacteria when they are lysed by CTLs where $N_C < N$.

The dynamics of the pathogen (which include extracellular bacteria (T_{BE}) and intracellular bacteria (T_{BI})) and immune mechanisms, the components being resting macrophages (M_R), infected macrophages (M_I), activated macrophages (M_A), CD4+ helper T cells (T), and CTLs (C), are modelled as follows.

2.5. **Model equations.**

2.5.1. *Macrophage dynamics.* The dynamics of macrophages populations (M_R, M_I, M_A) are modelled as follows:

$$\begin{aligned}\frac{dM_R(t)}{dt} &= \beta_m + \alpha_r\big(M_A(t) + \omega_r M_I(t)\big) + \sigma M_R(t)\Big(\frac{T_B(t)}{T_B(t)+S_R}\Big)\\ &\quad -\beta_i\Big(\frac{T_{BE}(t)M_R(t)}{T_{BE}(t)+S_E}\Big) - \alpha M_R(t) - \omega M_R(t)\Big(\frac{T_B(t)}{T_B(t)+S_A}\Big)\\ &\quad +\mu_d M_A(t) \qquad (1)\end{aligned}$$

$$\begin{aligned}\frac{dM_I(t)}{dt} &= \beta_i\Big(\frac{T_{BE}(t)M_R(t)}{T_{BE}(t)+S_E}\Big) - k_2 M_I(t) - k_3 M_I(t)\Big(\frac{C(t)}{C(t)+G_n}\Big)\\ &\quad -k_4\Big(\frac{M_I(t)}{1+b_0 T_{BI}(t)}\Big)\Big(\frac{T(t)}{T(t)+A_T}\Big) - k_5 M_I(t)C(t) - \mu_{MI} M_I(t) \qquad (2)\end{aligned}$$

$$\frac{dM_A(t)}{dt} = \omega M_R(t)\Big(\frac{T_B(t)}{T_B(t)+S_A}\Big) - \mu_a M_A(t) - \mu_d M_A(t). \qquad (3)$$

2.5.2. *Bacteria dynamics.* The dynamics of bacteria populations (T_{BI}, T_{BE}) are modelled as follows:

$$\begin{aligned}\frac{dT_{BE}(t)}{dt} &= Nk_2 M_I(t) + k_4 N_T\Big(\frac{M_I(t)}{1+b_0 T_{BI}(t)}\Big)\Big(\frac{T(t)}{T(t)+A_T}\Big) + k_5 N_C M_I(t)C(t)\\ &\quad +\gamma_4 T_{BE}(t) - \beta_i N_1 M_R(t)\Big(\frac{T_{BE}(t)}{T_{BE}(t)+S_E}\Big) - \gamma_5 T_{BE}(t)\Big(\frac{C(t)}{C(t)+G_n}\Big)\\ &\quad -\gamma_1 T_{BE}(t)M_A(t) - \gamma_2 T_{BE}(t)M_R(t). \qquad (4)\end{aligned}$$

$$\begin{aligned}\frac{dT_{BI}(t)}{dt} &= \varphi N M_I(t)\Big(1-\frac{T_{BI}^h(t)}{T_{BI}^h(t)+(NM_I(t))^h+E}\Big)-k_2NM_I(t)\\ &\quad -k_3N_2M_I(t)\Big(\frac{C(t)}{C(t)+G_n}\Big)+\beta_iN_1M_R(t)\Big(\frac{T_{BE}(t)}{T_{BE}(t)+S_E}\Big)\\ &\quad -k_4N_T\Big(\frac{M_I(t)}{1+b_0T_{BI}(t)}\Big)\Big(\frac{T(t)}{T(t)+A_T}\Big)-k_5N_CM_I(t)C(t)-\mu_gT_{BI}(t)\end{aligned} \tag{5}$$

2.5.3. *T cells dynamics.* The dynamics of Helper T cells and CTLs is modelled as follows:

$$\frac{dT(t)}{dt} = S_1+p_1\Big(\frac{M_A(t)+\alpha_TM_I(t)}{M_A(t)+\alpha_TM_I(t)+S_T}\Big)T(t)-\mu_TT(t) \tag{6}$$

$$\frac{dC(t)}{dt} = S_2+p_2\Big(\frac{(M_A(t)+\alpha_TM_I(t))T(t)C(t)}{M_A(t)+\alpha_TM_I(t)+S_C}\Big)-\mu_CC(t). \tag{7}$$

Equation (1) describes the dynamics of resting macrophages. The first term on the right-hand side of this equation represents the source of macrophages from monocyte differentiation from promonocytes from bone marrow at rate β_m. Alveolar macrophages are phagocytes that are specialized at antigen intake and adapted to the environment. Macrophages get adapted to the environment of the lungs. The second term is the additional recruitment of resting macrophages. These macrophages are recruited to the site of infection in the lungs in response to chemokines released by activated and infected macrophages at rates α_r and $\alpha_r\omega_r$ [11], respectively, where $(0<\omega_r<1)$. The third term is a recruitment term [11] for resting macrophages at rate σ, and this depends on the density of the bacterial load at the site of infection. Here S_R is the bacterial saturation constant for resting macrophages. The fourth term is an infection term for resting macrophages. Resting macrophages engulf extracellular bacteria, and when they fail to clear their bacterial load they become chronically infected, at rate β_i. Infection of resting macrophages depends on the density of extracellular bacteria present in the lungs; S_E is the saturation constant of extracellular bacteria. The fifth term is a natural death term for resting macrophages at rate α. The sixth term is a loss term to the population of resting macrophages when they get activated. This gives a set of cells called activated macrophages which are efficient at antigen killing. Activation is a result of stimulation due to IFN-γ, antigen and other cytokines at maximal rate of ω. S_A is the saturation constant of activated macrophages. Activated macrophages may also get deactivated by IL-10 at rate μ_d (seventh term).

Equation (2) describes the dynamics of infected macrophages. The first term on the right-hand side of this equation is a source term for infected macrophages from the infection term of equation (1) (term four of equation (1)). The second term is a loss term for infected macrophages due to bursting. Infected macrophages burst as a result of increase in bacterial burden. As bacteria multiply inside the cells, a threshold limit is reached; then bursting occurs, releasing bacteria to the extracellular environment. The third term represents the intracellular bacterial killing by antimicrobial effects of CTLs [13, 14], at rate k_3. This depends on the density of CTLs present at the site of infection, and G_n is the half maximum stimulation

of CTLs. The term $(\frac{M_I C}{C+G_n})$ captures the effects of CTLs in extracellular and intracellular killing of Mtb. Intracellular bacteria are killed through the peforin pole by granulysin [14]. One mechanism by which immunity arises has been postulated to be the lysis of infected cells by the antigen-specific CTLs [13]. This lysis is thought to be followed by the release of live bacteria, which are subsequently taken up and killed by newly immigrated and freshly activated macrophages [20]. However, the increasing bacterial burden in the cells will eventually cause spontaneous lysis, which raises the intriguing question of why CTL-mediated lysis of the cell would be beneficial for the host. The analysis of CTLs [13] showed that CTLs have the capacity to kill intracellular microbial pathogen directly. The study [13] showed that granulysin is a critical effector molecule of the antimicrobial activity of CTLs (which is found in the cytotoxic granules of T cells) [14]. The fourth term is apoptotic death of chronically infected macrophages induced by CD4+ helper T cells at rate k_4. Apoptotic death results in the release of bacteria. These bacteria may either infect resting macrophages or be killed by activated macrophages and exocytotic effects of granzymes produced by CD8+ T cells. Conversely, intracellular mycobacteria appear to have the ability to down-regulate apoptosis of their host macrophages, which may prolong their survival within the protective intracellular environment [11, 12]. The factor $\left(\frac{1}{1+b_0 T_{BI}}\right)$ represents the inhibition due to intracellular bacteria which hinders apoptosis. As bacterial burden increases, the infected macrophages are likely to die due to bursting rather than through apoptotic death, and b_0 is an inhibition factor which measures the efficiency with which intracellular bacteria (maximal effect of T_{BI} inhibition) inhibits apoptosis of infected macrophages. The fifth term represents lytic death of chronically infected macrophages due to CTL action. The last term presents the natural death of infected macrophages at a rate μ_{MI}.

Equation (3) describes the dynamics of activated macrophages. The first term on the right-hand side of this equation is the supply term for activated macrophages. A set of hormone-like chemical messengers called cytokines are produced, which in turn trigger the activation of resting macrophages. This gives a set of activated macrophages which are efficient at killing the bacteria at a rate ω. This is dependent on the bacterial density ($T_B = T_{BE} + T_{BI}$) (a gain term from the sixth term in equation (1)). The activated macrophages die naturally at a rate μ_a (second term). The cytokine environment affects the regulation of the immune response at the site of infection, slowing down macrophage activation under the action of $IL-4$ [28, 29] and facilitating macrophage deactivation ($IL-10$ down-regulation), at a rate μ_d (third term).

Equation (4) describes the dynamics of extracellular bacteria (T_{BE}). The first term on the right-hand side of this equation is the source term for extracellular bacteria. Mtb bacteria favors intracellular multiplication, but when the intracellular population reaches the maximum carrying capacity of N, infected macrophages [11] burst, releasing a total of NM_I bacteria at rate k_2. The second and third terms represent the exchange of bacteria from the intracellular environment to the extracellular due to killing of infected macrophages by CD4+ helper T and CTLs at rates k_4 and k_5, respectively, as captured in terms four and five of equation (2). We assume that CD4+ T cell-induced apoptotic macrophage death results in the release of N_T bacteria, while the lytic death due to CTLs releases N_C bacteria from infected macrophages. The fourth term represents multiplication of extracellular bacteria at rate γ_4. The fifth term models the amount of bacteria engulfed by

macrophages which manages to cause chronic infection at rate β_i, which is the rate at which extracellular bacteria interact with resting macrophages (infection term of equation (1)). N_1 is the amount of bacteria which resting macrophages accumulate to become chronically infected after failing to clear their bacterial load ($N_1 = \frac{N}{2}$) [11]. The sixth term represents the extracellular bacteria killing by granule components of CTLs at rate γ_5. This action is increased as CTL density increases. The seventh and eight terms are the bacteria killing by activated and resting macrophages at rates γ_1 and γ_2 respectively. Wigginton and Kirschner [11] used similar terms to model direct killing of extracellular bacteria.

Equation (5) describes the dynamics of intracellular bacteria. The first term on the right-hand side of this equation describes the intracellular bacteria multiplication and growth according to Hill's equation, which describes a sigmoidal curve [11]. Here h is called the Hill coefficient, and NM_I represents the concentration or amount of intracellular bacterial particles inside infected macrophages. The value of Hill coefficient gives a measure of the degree of bacterial multiplication and regulates the shape of the function (from gently sloping to step function) [1, 11]. This process takes place at rate φ, and E is a small constant, which keeps the Hill's function bounded from zero [11]. The second term is a loss term as intracellular bacteria are released to the extracellular environment due to bursting at rate k_2. The third term represents the intracellular killing of bacteria by CTLs. As density of CTLs increases, the intracellular bacteria population decreases. N_2 is the total number of intracellular bacteria in a chronically infected macrophage killed as a result of CTL intracellular killing. The fourth term is a gain term for intracellular bacteria at a maximal rate of β_i. This is a result of resident macrophages engulfing extracellular bacteria, failing to remove their bacterial particles, and then becoming chronically infected. The number of bacterial particles which are added to the intracellular environment depends on the population or density of extracellular bacteria which interact with resident macrophages. As T_{BE} increases, the intake factor is enhanced. The fifth term represents loss of bacterial particles, which are released due to induced apoptotic death by CD4+ helper T cells. This takes place at a maximal rate k_4 where N_T is the number or amount of bacteria released as a result of macrophage programmed death due to CD4+ helper T cells. The sixth term represents the loss of N_C bacterial particles at rate k_5 from each infected macrophage lysed by CTLs. As the density of CTLs increase, a substantial number of infected macrophages are lysed. The last term represents the loss of intracellular bacteria caused by macrophage activities, which kill intracellular bacteria at rate μ_g. When bacterial particles are phagocyzed in a phagosome, the particles are killed when phagosome-lysosome fusion occurs. Macrophages have mechanisms that kill intracellular bacteria, such as phagosome-lysosome fusion and nitrogen and oxygen reactive intermediates [18, 27].

Equation (6) describes the dynamics of CD4+ helper T cells. The first term on the right-hand side of this equation is a source term for CD4+ T cells from thymus. Due to antigen detection, cytokines are released to differentiate Th0 precursor cells into Th1 response or Th2 response, and this differentiation depends on the combination of cytokines released. Th1 is postulated to save as a preventive measure in which IFN-γ and IL-12 are dominant (Th1 response is associated with control of the infection), and Th2 is evident in active TB with IL-4, 5, and 6 being dominant cytokines. T cells in this work are given by the total of T cells in Th1 and Th2 immune responses. The second term is the proliferation and recruitment

term for CD4+ helper T cells at rate p_1 due to cytokines released by infected and activated macrophages. This proliferation depends on the density of infected and activated macrophages. The third term represents the death of CD4+ helper T cells at rate μ_T. S_T is the half maximum saturation constant of cells which stimulate recruitment and proliferation of CD4+ helper T cells.

Equation (7) describes the dynamics of CD8+ T cytotoxic cells. S_2 is the supply term for CD8+ T cells from the thymus. The second term is a proliferation term for CTLs which depends on the number of infected and activated macrophages, which secrete cytokines which trigger the cell mediated immune response. It also depends on the count of CD4+ T cells. If the population of CD4+ T cells declines the CTL count also declines [25]. This decline was also noticed in patients infected with HIV [21]. The last term represents the natural death of CTLs at rate μ_C. S_C is the half maximum saturation constant of cells which stimulate recruitment and proliferation of CTLs.

3. **Model analysis.** At the disease-free state, no bacteria are present and as a result there are no infected macrophages, activated macrophages, extracellular or intracellular bacteria. At the disease free-state, populations of all species involved in the immune interactions are given by

$$(M_R^*, M_I^*, M_A^*, T_{BE}^*, T_{BI}^*, T^*, C^*) = (\frac{\beta_m}{\alpha}, 0, 0, 0, 0, \frac{S_1}{\mu_T}, \frac{S_2}{\mu_C}). \tag{8}$$

At the endemically infected state, macrophages become chronically infected, and this triggers activation of resting macrophages. The equlibrium states at this endemically infected state represent two possible disease outcomes, that is, latency and primary disease. The occurrence of latent infection depends on a set of parameters, and when these parameters vary primary disease occurs. Chronically infected macrophages burst, releasing intracellular bacteria into the extracellular environment, and recruitment of CD4+ helper T cells and CTLs to the site of infection occurs.

The endemically infected state is given by

$$\bar{F} = (M_R^*, M_I^*, M_A^*, T_{BE}^*, T_{BI}^*, T^*, C^*), \tag{9}$$

where $M_R^*, M_I^*, T_{BE}^*, T_{BI}^*, T^*$ and C^* are given by the expressions (10, 11, 12, 16, 22, 23, and 24) as follows.

The equilibrium value for resting macrophages at the endemically infected state is given by

$$M_R^* = \frac{(\beta_m + \alpha_r(M_A^* + \omega_r M_I^*) + \mu_d M_A^*)}{(\frac{\beta_i T_{BE}^*}{T_{BE}^* + S_E} + \alpha + \frac{\omega T_B^*}{T_B^* + S_A} - \frac{\sigma T_B^*}{T_B^* + S_R})}. \tag{10}$$

The numerator of expression (10) shows that activated and infected macrophages prompt an increase and recruitment of more resting macrophages. The denominator shows that an increase in intracellular and extracellular bacteria has a negative impact on the increase of resting macrophages population. As both bacterial populations approach zero, the resting macrophages return to their steady supply of $\frac{\beta_m}{\alpha}$.

At the endemically infected state, the population of infected macrophages is given by

$$M_I^* = \frac{\beta_i M_R^* T_{BE}^*}{(T_{BE}^* + S_E)(k_2 + \frac{k_3 C^*}{C^* + G_n} + \frac{k_4 T^*}{(1 + b_o T_{BI}^*)(T^* + A_T)} + k_5 C^* + \mu_{MI})}. \tag{11}$$

This expression shows that the interaction of resting macrophages and extracellular bacteria supports the increase of infected macrophages. The density of CTLs and CD4+ helper T cells, and the bursting and death rate of infected macrophages oppose the increase in population of infected macrophages. The increase in the bursting of infected macrophages (k_2) and death (μ_{MI}) rate for infected macrophages also results in a decrease in M_I population.

The expression for activated macrophages at the endemically infected state is given by

$$M_A^* = (\frac{\omega}{\mu_a+\mu_d})\frac{T_B^* M_R^*}{T_B^*+S_A}. \tag{12}$$

From this expression we notice that during chronic TB infection an increase in the populations of both intracellular and extracellular bacteria prompts the activation of macrophages, since as $T_B^* \to 0$, $\frac{T_B^*}{T_B^*+S_A} \to 0$ and as $T_B^* \to \infty$, $\frac{T_B^*}{T_B^*+S_A} \to 1$.

The equilibrium value of extracellular bacteria is evaluated from

$$a_2 T_{BE}^{*^2} + a_1 T_{BE}^* + a_0 = 0, \tag{13}$$

where

$$\begin{aligned} a_0 &= S_E N k_2 + \frac{S_E k_4 N_T M_I^* T^*}{(1+b_0 T_{BI}^*)(T^*+A_T)} + S_E k_5 N_C M_I^* C^* \\ a_1 &= \gamma_4 S_E - \beta_i N_1 M_R^* - \frac{\gamma_5 S_E C^*}{C^*+G_n} - \gamma_1 S_E M_A^* - \gamma_2 S_E M_R^* + N k_2 \\ &\quad + \frac{k_4 N_T M_I^* T^*}{(1+b_0 T_{BI}^*)(T^*+A_T)} + k_5 N_C M_I^* C^* \\ a_2 &= \gamma_4 - \frac{\gamma_5 C^*}{C^*+G_n} - \gamma_1 M_A^* - \gamma_2 M_R^*. \end{aligned} \tag{14}$$

Therefore T_{BE}^* is given by

$$T_{BE}^* = \frac{-a_1 \pm \sqrt{a_1^2 - 4a_2 a_0}}{2a_2}. \tag{15}$$

That is, either

$$T_{BE_1}^* = \frac{-a_1 - \sqrt{a_1^2 - 4a_2 a_0}}{2a_2}$$

or

$$T_{BE_2}^* = \frac{-a_1 + \sqrt{a_1^2 - 4a_2 a_0}}{2a_2}.$$

Note: a_1 can be expressed as $a_1 = a_2 S_E + \frac{a_0}{S_E} - \beta_i N_1 M_R$. If $a_1 < 0$ then it follows that $a_2 S_E < \beta_i N_1 M_R - \frac{a_0}{S_E}$, but $\beta_i N_1 M_R - \frac{a_0}{S_E} < 0$ and it follows that $a_2 < 0$. That is if $a_1 < 0$, then also $a_2 < 0$. Now if $a_1 > 0$, it follows that either $a_2 < 0$ or $0 < a_2$, but a_2 can never be greater than zero, since the condition $0 < a_2$ violates the stability condition (i) of expression (25).

Therefore the biological value of extracellular bacteria is always greater than or equal to zero and is given by

$$T_{BE}^* = T_{BE_2}. \tag{16}$$

We deduce from (14) that the value of extracellular bacteria depends mainly on the value of γ_4, its ability to multiply outside macrophages, and the direct killing of the bacteria by CTL-related action and activated macrophages. The more the scavenger

macrophages present at the site of infection, the less the number of extracellular bacteria at the site of infection.

The equilibrium value for intracellular bacteria is evaluated from

$$T_{BI}^{*^3} + \lambda_2 T_{BI}^{*^2} + \lambda_1 T_{BI}^{*} + \lambda_0 = 0, \tag{17}$$

where

$$\begin{aligned}\lambda_0 &= \frac{1}{\mu_g}\big((NM_I)^2 + E\big)\Big(k_2 NM_I + k_3 N_2 M_I\big(\frac{C}{C+G_n}\big) \\ &\quad + k_4 N_T\big(\frac{M_I}{1+b_o T_{BI}}\big)\big(\frac{T}{T+A_T}\big) \\ &\quad + k_5 N_C M_I C - \beta_i N_1 M_R\big(\frac{T_{BE}}{T_{BE}+S_E}\big) - \varphi N M_I\Big) \qquad (18)\\ \lambda_1 &= (NM_I)^2 + E \\ \lambda_2 &= \frac{1}{\mu_g}\Big(k_2 NM_I + k_3 N_2 M_I\big(\frac{C}{C+G_n}\big) + k_4 N_T\big(\frac{M_I}{1+b_o T_{BI}}\big)\big(\frac{T}{T+A_T}\big) + k_5 N_C \\ &\quad - \beta_i N_1 M_R\big(\frac{T_{BE}}{T_{BE}+S_E}\big)\Big). \qquad (19)\end{aligned}$$

Using the cubic formulae, the equilibrium value of intracellular bacteria is given by

$$\begin{aligned} T_{BI_1}^{*} &= -\frac{1}{3}\lambda_2 + (H+O) \\ T_{BI_2}^{*} &= -\frac{1}{3}\lambda_2 - \frac{1}{2}(H+O) - \frac{1}{2}\sqrt{3(O-H)} \\ T_{BI_3}^{*} &= -\frac{1}{3}\lambda_2 - \frac{1}{2}(H+O) + \frac{1}{2}\sqrt{3(O-H)}, \end{aligned} \tag{20}$$

where

$$\begin{aligned} H &= \sqrt[3]{R+\sqrt{D}}, \quad O = \sqrt[3]{R-\sqrt{D}}, D = Q^3 + R^2 \\ Q &= \tfrac{3\lambda_1 - \lambda_2^2}{9}, \qquad R = \frac{9\lambda_1\lambda_2 - 27\lambda_0 - 2\lambda_2^3}{54}. \end{aligned} \tag{21}$$

Three possible values may represent the value of intracellular bacteria at the endemically infected state; these are mathematically correct, but biologically not all of them are feasible. We take the positive value only. Therefore

$$T_{BI}^{*} = T_{BI_1}^{*}. \tag{22}$$

The number of bacteria inside infected macrophages depends on the ability of the pathogen to persist inside the infected macrophages irrespective of immune response mechanisms and is greatly reduced by intracellular killing of bacterial particles by CTL components and lytic, as well as by apoptotic killing by CD4+ helper T cells.

At the endemically infected state, the equilibrium value for CD4+ helper T cells is given by

$$T^{*} = \frac{S_1(M_A^{*} + \alpha_T M_I^{*}) + S_1 S_T}{(\mu_T - p_1)(M_A^{*} + \alpha_T M_I^{*}) + \mu_T S_T}. \tag{23}$$

We notice from expression (23) that as the number of infected macrophages increases the number of activated macrophages also increases, and the proliferation of CD4+ helper T cells increases to a maximum constant value of $(\frac{S_1}{\mu_T - p_1})$, which depends on the proliferation rate p_1 and the death rate μ_T.

The endemically infected equilibrium value of CD8+ T cells is given by

$$C^* = \frac{S_2(M_A^* + \alpha_T M_I^*) + S_2 S_C}{(\mu_C - p_2 T^*)(M_A^* + \alpha_T M_I^*) + \mu_C S_C}. \tag{24}$$

The value of CD8+ specific T cells depends on the number of infected and activated macrophages, which also depends on M_R and T_B, primarily because these cells are responsible for triggering and stimulation of all cytokines collectively responsible for mounting an adaptive immune response. These cytokines in turn stimulate CD8+ T cells. It also depends on the CD4+ T cell population, which is supportive to CD8+ T cell population growth. The decline in CD4+ T cells results in the decline of CD8+ T cells and hence progression of disease as noticed in HIV patients [21].

4. **Reproductive ratio.** In the case of TB infection, the reproductive ratio is the number of newly infected macrophages that arise from one infected macrophage when almost all macrophages are uninfected. When the reproduction ratio is less than one ($R_0 < 1$), infection is abortive, and when the reproduction ratio is greater than one ($R_0 > 1$), infection persists to active disease or latent infection. The main thrust in controlling infection is to reduce R_0 to a value less than unity; an accurate measurement of R_0 will reflect the true rate at which the infection grows, hence proposing the degree of chemotherapy use.

The Jacobian matrix for system (1) to (7) evaluated at the uninfected state is given by

$$J = \begin{pmatrix} -\alpha & \alpha_1 & \alpha_2 & \alpha_3 & \alpha_4 & 0 & 0 \\ 0 & \alpha_7 & 0 & \alpha_8 & 0 & 0 & 0 \\ 0 & 0 & \alpha_9 & \alpha_{10} & \alpha_{11} & 0 & 0 \\ 0 & \alpha_{12} & 0 & \alpha_{13} & 0 & 0 & 0 \\ 0 & \alpha_{14} & 0 & \alpha_{15} & \alpha_{16} & 0 & 0 \\ 0 & \alpha_{17} & \alpha_{18} & 0 & 0 & \alpha_{19} & 0 \\ 0 & \alpha_{20} & \alpha_{21} & 0 & 0 & 0 & \alpha_{22} \end{pmatrix},$$

where

$$\begin{aligned}
&\alpha_1 = \alpha_r \omega_r, && \alpha_2 = \alpha_r + \mu_d \\
&\alpha_3 = \tfrac{\sigma \bar{M}_R}{S_R} - \tfrac{\beta_i \bar{M}_R}{S_E} - \tfrac{\omega \bar{M}_R}{S_A}, && \alpha_4 = \tfrac{\sigma \bar{M}_R}{S_R} - \tfrac{\omega \bar{M}_R}{S_A} \\
&\alpha_7 = -k_2 - \tfrac{k_3 \bar{C}}{\bar{C}+G_n} - \tfrac{k_4 \bar{T}}{\bar{T}+A_T} - k_5 \bar{C} - \mu_{MI}, && \alpha_8 = \tfrac{\beta_i \bar{M}_R}{S_E} \\
&\alpha_9 = -(\mu_A + \mu_d), && \alpha_{10} = \tfrac{\omega \bar{M}_R}{S_A} \\
&\alpha_{11} = \tfrac{\omega \bar{M}_R}{S_A}, && \alpha_{12} = Nk_2 + \tfrac{k_4 N_T \bar{T}}{\bar{T}+A_T} + k_5 N_C \bar{C} \\
&\alpha_{13} = \gamma_4 - \tfrac{\beta_i N_1 \bar{M}_R}{S_E} - \tfrac{\gamma_5 \bar{C}}{\bar{C}+G_n} - \gamma_2 \bar{M}_R, && \\
&\alpha_{14} = \varphi N - k_2 N - \tfrac{k_3 N_2 \bar{C}}{\bar{C}+G_n} - \tfrac{k_4 N_T \bar{T}}{\bar{T}+A_T} - k_5 N_C \bar{C} && \\
&\alpha_{15} = \tfrac{\beta_i N_1 \bar{M}_R}{S_E}, && \alpha_{16} = -\mu_g \\
&\alpha_{17} = \tfrac{p_1 \alpha_T \bar{T}}{S_T}, && \alpha_{18} = \tfrac{p_1 \bar{T}}{S_T} \\
&\alpha_{19} = -\mu_T, && \alpha_{20} = \tfrac{p_2 \alpha_T \bar{T} \bar{C}}{S_C}, \\
&\alpha_{21} = \tfrac{p2 \bar{T} \bar{C}}{S_C}, && \alpha_{22} = -\mu_C.
\end{aligned}$$

The eigenvalues of the Jacobian matrix J can be determined by solving the characteristic equation $|J - \lambda I| = 0$; that is,

$$(-\alpha - \lambda)(\alpha_9 - \lambda)(\alpha_{19} - \lambda)(\alpha_{22} - \lambda)(\alpha_{16} - \lambda)\Big(\alpha_7 - \lambda)(\alpha_{13} - \lambda) - \alpha_8 \alpha_{12}\Big) = 0.$$

This gives $\lambda_1 = -\alpha, \lambda_2 = \alpha_9, \lambda_3 = \alpha_{19}$, $\lambda_4 = \alpha_{16}, \lambda_5 = \alpha_{22}$, and $\lambda^2 - (\alpha_{13} + \alpha_7)\lambda + \alpha_7\alpha_{13} - \alpha_8\alpha_{12} = 0$, and for stability $\lambda_1, \lambda_2, \lambda_3$, λ_4 and λ_5 must be less than zero; that is, $-\alpha < 0, \alpha_9 < 0, \alpha_{19} < 0, \alpha_{22} < 0, \alpha_{16} < 0$. Routh Hurwitz criteria for stability require that

$$\begin{aligned} (i) \quad & -(\alpha_7 + \alpha_{13}) > 0 \\ (ii) \quad & (\alpha_7\alpha_{13} - \alpha_8\alpha_{12}) > 0. \end{aligned} \tag{25}$$

Because α_7 and α_{13} are negative when $\frac{\beta_i N_1 \bar{M}_R}{S_E} + \frac{\gamma_5 \bar{C}}{\bar{C}+G_n} + \gamma_2 \bar{M}_R > \gamma_4$, (i) is satisfied and (ii) implies that $\left(\frac{\alpha_8\alpha_{12}}{\alpha_7\alpha_{13}}\right) < 1$. Therefore it follows that $R_0 = \left(\frac{\alpha_8\alpha_{12}}{\alpha_7\alpha_{13}}\right)$. Putting the values of $\alpha_7, \alpha_8, \alpha_{12}, \alpha_{13}$ into the expression for R_0 gives

$$R_o = \frac{\frac{\beta_i \bar{M}_R}{S_E}\left(Nk_2 + \frac{k_4 N_T \bar{T}}{\bar{T}+A_T} + k_5 N_C \bar{C}\right)}{\left(k_2 + \frac{k_3 \bar{C}}{\bar{C}+G_n} + \frac{k_4 \bar{T}}{\bar{T}+A_T} + k_5 \bar{C} + \mu_{MI}\right)\left(\frac{\beta_i N_1 \bar{M}_R}{S_E} + \frac{\gamma_5 \bar{C}}{\bar{C}+G_n} + \gamma_2 \bar{M}_R - \gamma_4\right)}. \tag{26}$$

For R_0 to be positive,

$$\frac{\beta_i N_1 \bar{M}_R}{S_E} + \frac{\gamma_5 \bar{C}}{\bar{C}+G_n} + \gamma_2 \bar{M}_R > \gamma_4. \tag{27}$$

Note: This is also the condition (i) of expression (25) required for stability.

The reproductive rate is dependent on several factors ($\beta_i, \beta_m, \mu_C, S_2, G_n, \mu_T, A_T, \alpha, k_2, k_4, N, N_T, k_5, N_C, \gamma_4, N_1, \gamma_2, \gamma_5$). The rate of bacterial multiplication outside the macrophages has an impact on the overall rate of disease progression. We notice from (26) that the smaller the value of γ_4, the bigger the reproductive number. The value of the reproductive ratio depends significantly not only on the value of γ_4 but also on the lytic killing of infected macrophages. The direct bacteria killing by CTLs also significantly affects the reproduction ratio. The ability of CTLs to lyse infected macrophages and to kill both intracellular and extracellular bacteria reduces the rate of progression of the disease. We also deduce from expression (26) that the rate at which infected macrophages burst and die and the rate at which resting macrophages are infected can influence the progression of the disease.

5. **Numerical simulations.** We solve the system of ordinary differential equations (1), (2), (3), (4), (5), (6), and (7) numerically by using the C code based on Runge-kutta Order Four method. Rates of interactions and kinetics are estimated from published experimental data. Human-derived experimental data and non-human primate (NHP) data are used in estimations where possible [12]. Other animal data (mouse, rabbit) are used to derive magnitude estimates when no human or primate data are available [12]. The following initial conditions were used in the numerical simulations: $M_R(0) = 100000$, $T_{BE}(0) = 2000$, $M_I(0) = 0$, $M_A(0) = 0$, $T_{BI}(0) = 0$, $T(0) = 500$ and $C(0) = 140$. In all our simulations we used a time step (h) of size 0.5. The parameters used in the numerical simulations are in Table 1 below. In obtaining different numerical results, that is latency and primary disease only the value of γ_4 was varied.

TABLE 1. Table of Parameters used in the model.

Name	Value	Definition	Units	Reference
β_m	5000	M_R source	$M_R cm^{-3} day$	[12]
α_r	0.05	Recruitment due to M_A	day^{-1}	[12]
ω_r	0.4	Recruitment due to M_I	scalar	[12]
σ	0.01	Proliferation of M_A	day^{-1}	[12]
S_R	1000000	Saturation limit of T_B	$T_B cm^{-3}$	[12]
β_i	0.4	Infection rate	day^{-1}	[12]
α	0.011	Death	day^{-1}	[12]
ω	0.03	Maximal activation	day^{-1}	Estimated
S_A	500000	Sat constant of activation M_R	$T_B cm^{-3}$	[12]
k_2	0.4	Bursting	day^{-1}	[12]
μ_{MI}	0.011	Death	day^{-1}	[12]
μ_a	0.011	Death	day^{-1}	[12]
N	50	Burst Size	$T_{BI} M_I^{-1}$	[12]
N_1	25	T_{BE} size that cause chronic infection	$T_{BI} M_I^{-1}$	[12]
γ_1	0.000000125	T_{BE} killing by M_A	$(cm^3 M_I^{-1}) day$	[12]
γ_2	0.0000000125	T_{BE} killing by M_R	$(cm^3 M_I^{-1}) day$	[12]
γ_4	0.1	T_{BE} multiplication	day^{-1}	[12]
φ	0.49	T_{BI} multiplication	day^{-1}	[12]
S_1	100	source of T	day^{-1}	Estimated
S_E	1000000	T_{BE} saturation limit	$T_{BE} cm^{-3}$	[12]
μ_d	0.3	M_A deactivation	day^{-1}	Estimated
k_3	0.000000125	T_{BI} killing by C (CTL granules)	day^{-1}	Estimated
N_T	40	T_{BE} size killed by T	$T_{BI} M_I^{-1}$	Estimated
N_C	40	T_{BE} size killed by C	$T_{BI} M_I^{-1}$	Estimated
N_2	30	T_{BI} size killed by C (CTL granules)	$T_{BI} M_I^{-1}$	Estimated
k_4	0.000000125	Apoptosis	day^{-1}	Estimated
b_o	500000	Apoptosis inhibition factor	scalar	Estimated
k_5	0.00000185	Lysis	day^{-1}	Estimated
γ_5	0.85	T_{BE} killing by C	day^{-1}	Estimated
S_2	100	source of C	C $cm^{-3} day^{-1}$	Estimated
S_T	1500000	T saturation Limit	T $cm^{-3} day^{-1}$	Estimated
A_T	1000	Half-sat of T for Apoptosis	$(cm^3 T^{-1}) day$	Estimated
α_T	0.3	Recruitment	day^{-1}	Estimated
p_1	0.03	Proliferation of T	day^{-1}	Estimated
p_2	0.01	Proliferation of C	day^{-1}	Estimated
μ_T	0.01	Death	day^{-1}	Estimated
μ_C	0.68	Death	day^{-1}	Estimated
E	10	Bounding value	scalar	Estimated
G_n	1000	CTL granules saturation constant	$(cm^3 T^{-1}) day$	Estimated
μ_g	0.011	T_{BI} killing M_R immune mechanisms	day^{-1}	Estimated
S_C	1500000	C saturation limit	$C cm^{-3} day^{-1}$	Estimated

5.1. **Latent infection.** The ability of the bacteria to persist inside macrophages and the inefficiency of the resting macrophages at killing the pathogen aids in developing latency. Also the ability of the Mtb to resist killing by toxic effects of phagosome-lysosomes and oxygen- and nitrogen-reactive intermediates as well as CTLs toxic effects results in latent infection. In modelling the occurance of latency we used a small value of γ_4. This is because the occurance of latency is a result of the ability of intracellular bacteria to persist inside macrophages.

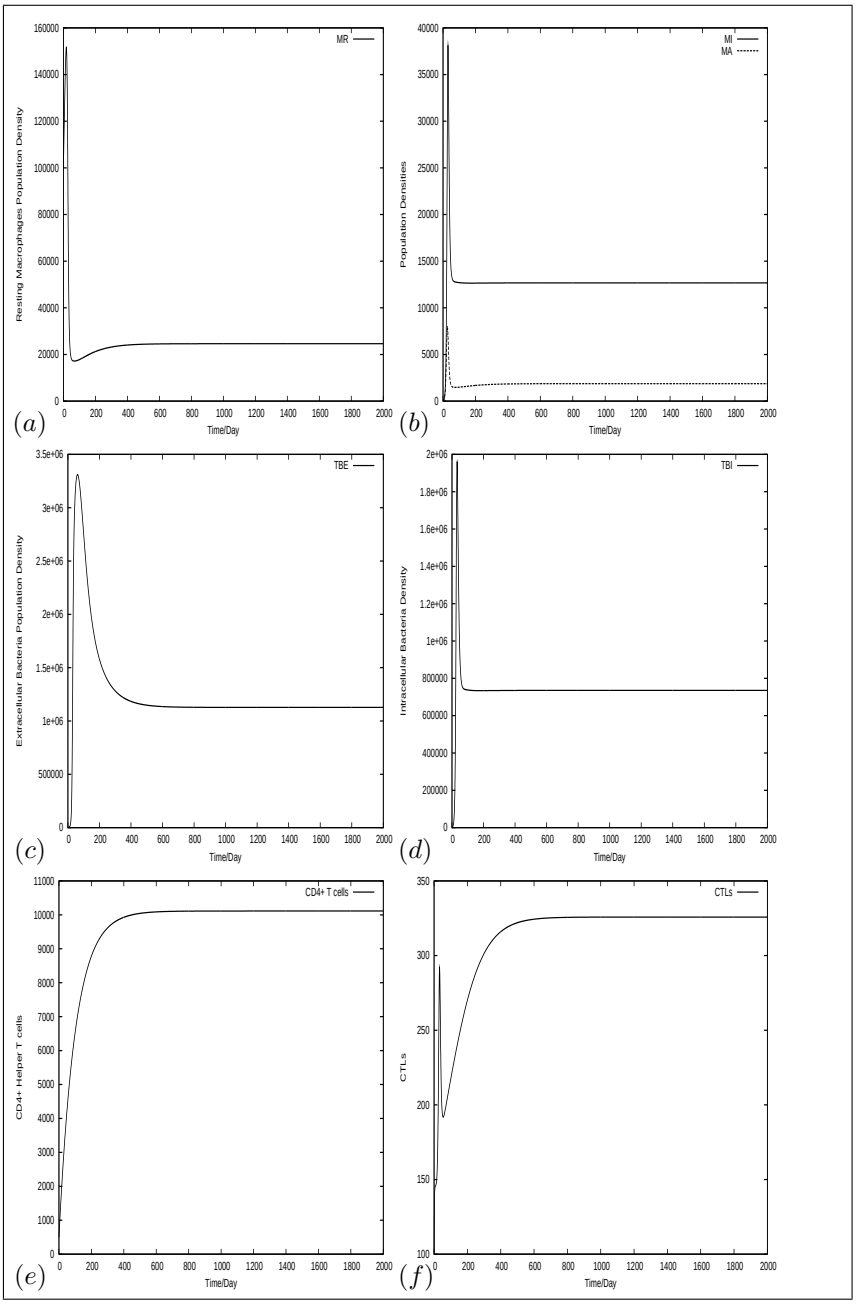

FIGURE 1. Graph of numerical solution showing propagation of macrophages, bacteria, and T cells during the first 2000 days: (a) time evolution of resting macrophages, (b) infected and activated macrophages kinetics during the first 2000 days of Mtb infection, (c) time population density changes of extracellular bacteria, (d) the change in population density of intracellular bacteria, (e) time evolution of CD4+ helper T cells, and (f) CTLs kinetics during Mtb latent infection ($\gamma_4 = 0.1$).

Figure 1 shows the changes in human immune response mechanisms with time during infection with Mtb. Figure 1(a) shows the time evolution of resting macrophages while Figure 1(b) shows the time evolution of activated and infected macrophages. We notice from this figure that the population of resting macrophages (M_R) increases as a result of the increasing population of infected macrophages (M_I) and activated macrophages (M_A). The graph shows that within about the first 100 days of infection the growth or increase in infected macrophages is controlled and maintained at a constant population. We also notice that M_A increases and falls rapidly within about the first 100 days. After about 100 days M_A increases steadily and reaches a constant steady level after 200 days, but the population of M_I persists as shown in Figure 1(b). Figures 1(c) and 1(d) show changes in population densities of extracellular and intracellular bacteria with time, respectively. Extracellular (T_{BE}) and intracellular (T_{BI}) bacteria go through rapid dynamical changes in about the first 200 days, for T_{BI} a steady state is achieved after 600 days. The drop in T_{BE} and T_{BI} corresponds to an increase in M_A and M_R, as illustrated in Figures 1(a) and (b). Figures 1(d) and 1(e) show changes in CD4+ helper T cells and CTLs with time during latent infection. CD4+ helper T cell count initially increases and is maintained at a constant level after after 400 days. The dynamics of CTLs changes rapidly in about the first 200 days. After 200 days CTLs increase until they reach a constant level, and this is attained after 600 days. We notice from Figures 1(c) and 1(f) that the decline in T_{BE} corresponds to the increase in CTLs. A steady constant level of CTLs is attained as the constant level of T_{BE} is being attained. Nevertheless the intracellular and extracellular bacteria persist as a result of M_I persistence which leads to latent infection.

5.2. **Primary disease.** Figure 2 shows changes in human immune response mechanisms with time, which results in primary disease. Figure 2(a) shows changes in the densities of resting macrophages, and Figure 2(b) shows time evolution of activated and infected macrophages. We notice that there is a rapid increase and change in the populations of M_A, M_I and M_R within about the first 100 days. The levels of M_R and M_I are approximately equal, and that of M_A is relatively low. These levels are maintained, but extracellular bacteria grows out of proportion after about 600 days as M_R, M_A, and other immune mechanisms involved fail to suppress the infection. Figure 2(c) and 2(d) show the time evolution of extracellular and intracellular bacteria, respectively. The T_{BI} population level is maintained at a constant level after about 100 days. This is a result of the constant level of M_I. T_{BE} population grows out of control after a protracted period of about 600 days. This explains why some individuals develop active disease many years (1-5 years) [12] after the time of infection. Figures 2(e) and 2(f) show the time evolution of CD4+ helper T cells and CTLs. The CD4+ T count increases and is maintained at a constant level. CTLs cell count increase and fall within about the first 100 days. After about the first 100 days, an increase in CTLs is noticed, and they reach a constant level after 600 days. A point of interest here is that in the progression to active disease shown in Figure 2, the levels of CTLs, M_A and M_R are lower than the levels of the same species shown in Figure 1 (which show progression to latent TB).

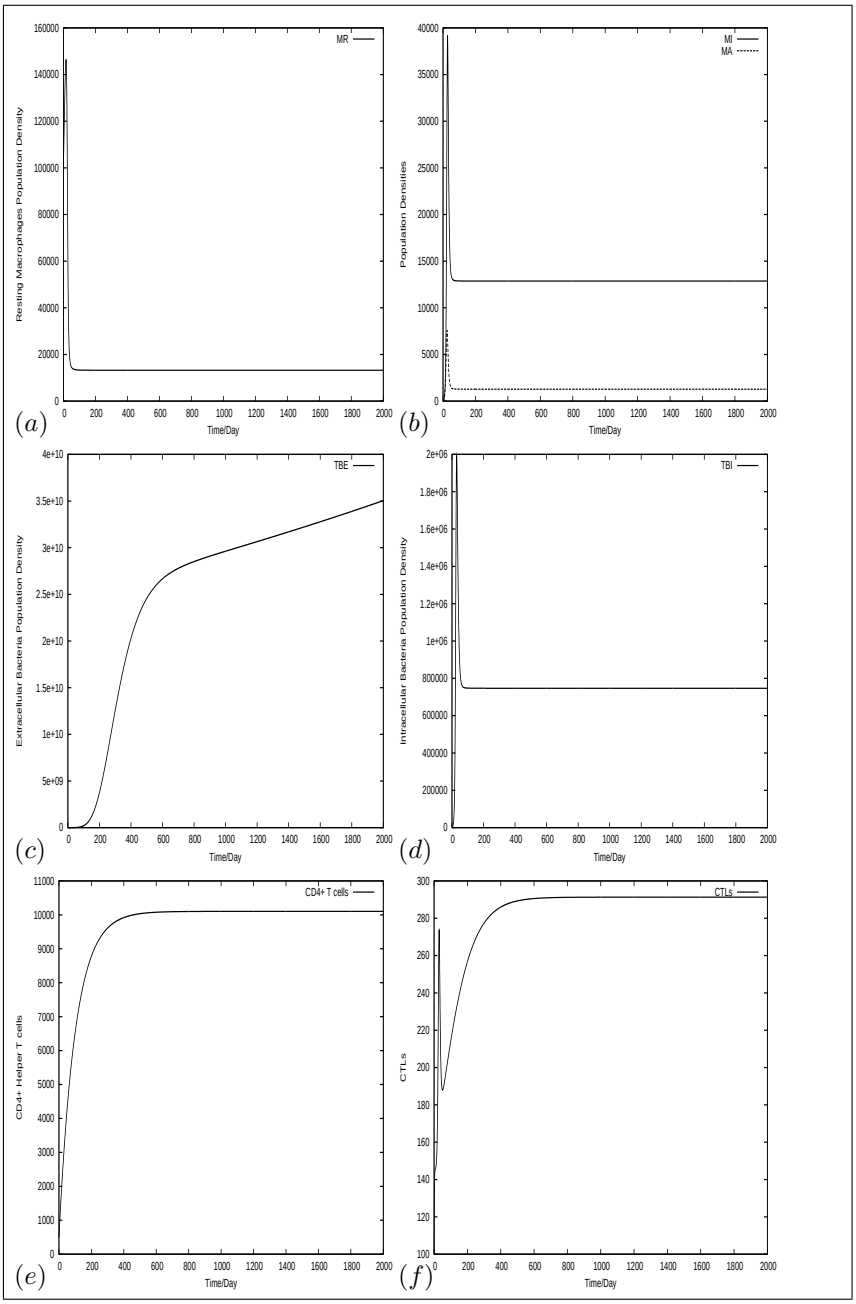

FIGURE 2. Graph of numerical solution showing propagation of macrophages, bacteria, and T cells during the first 2000 days: (a) time evolution of resting macrophages, (b) infected and activated macrophages kinetics during the first 2000 days of Mtb infection, (c) time population density changes of extracellular bacteria, (d) the change in population density of intracellular bacteria, (e) time evolution of CD4+ helper T cells, and (f) CTLs kinetics during Mtb latent infection ($\gamma_4 = 0.19225$).

5.3. **Effects of initial conditions.** In numerical simulations of the model, we also observed that the velocity of infection progression changes with changes in the initial bacterial load. Increasing the initial bacterial load gives a fast progression to either latency or primary TB. This suggests that the range of years within which individual's progress to active disease might depend on the initial dose as well as on other factors. The graph in Figure 3 shows how active disease is achieved, starting with different bacterial loads at the time of infection. TBE-1 shows velocity of progression to active disease when an initial bacterial dose of 20 is used, while TBE-2 and TBE-3 show the velocity of disease progression when initial bacterial loads of 2000 and 20000 are used, respectively.

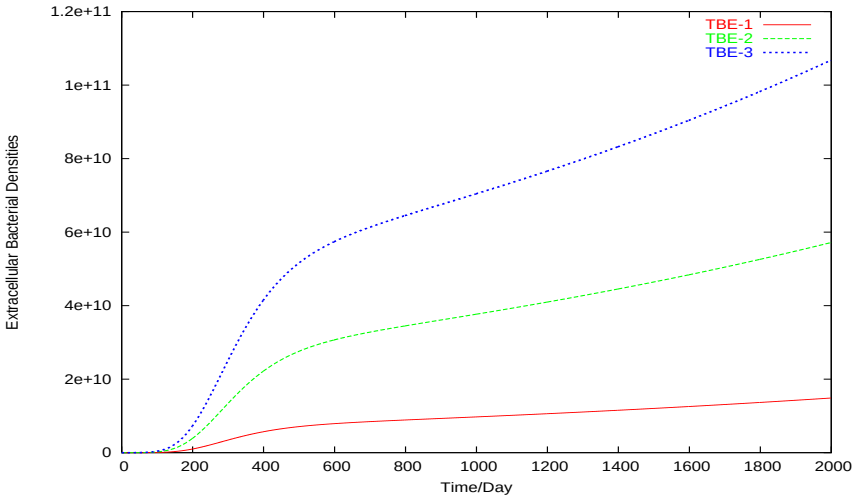

FIGURE 3. Graph of velocity of progression to active disease emanating from different initial bacterial doses or size at infection (TBE-1=20 bacterial particles, TBE-2=2000, and TBE-3=20000). The initial conditions used are $M_R(0) = 100000$, $M_I(0) = 0$, $M_A(0) = 0$, $T_{BI}(0) = 0$, $T(0) = 500$ and $C(0) = 140$ and $\gamma_4 = 0.1925$. All other parameters are as given in Table 1

5.4. **Uncertainty and sensitivity analysis.** We did uncertainty and sensitivity analysis to determine parameters which contribute to the variability of the disease outcome from a scenario that lead to active disease. We used the population of extracellular bacteria to quantify the effectiveness of perturbations of each parameter, since high levels of T_{BE} result in active TB.

Figure 4(a) shows that reducing the rate (k_2) at which infected macrophages burst results in a switch from active to latent TB. Figure 4 (b) shows that larger values of k_5 (rate of lytic killing of M_I by CTLs) favors exponential growth of extracellular bacteria and smaller value forces a drop from exponential level to levels which result in latent TB in an environment that favors extracellular bacteria multiplication. We also notice in Figure 4(c) that the ability of Mtb to multiply outside the protected environment of macrophages favors development of active disease. Here TBE-1 represents the smallest rate of T_{BE} multiplication, while TBE-6 represents the highest rate of multiplication. Another point to take note of

is the rate at which resting macrophages kill extracellular bacteria. Our analysis shows that if the rate at which M_R kills T_{BE} is high, then disease will not develop to active TB, as shown in Figure 4(d) (TBE-6 represents the highest rate of γ_2). Another critical parameter is γ_5; Figure 4(e) shows that higher values of γ_5 (CTL killing of intracellular and extracellular bacteria) result in the control of Mtb and its lower values lead to active TB.

TABLE 2. k_2, k_5, γ_2, γ_4 and γ_5 are the values used for uncertainty and sensitivity analysis in Figure 4.

Figure	Parameter value	TBE-key
Figure 4(a)	k_2	
	0.300	TBE-1
	0.385	TBE-2
	0.390	TBE-3
	0.395	TBE-4
	0.400	TBE-5
Figure 4(b)	k_5	
	0.000 000 018 5	TBE-1
	0.000 000 185 0	TBE-2
	0.000 001 850 0	TBE-3
	0.000 010 000 0	TBE-4
	0.000 011 850 0	TBE-5
Figure 4(c)	γ_4	
	0.18800	TBE-1
	0.19000	TBE-2
	0.19100	TBE-3
	0.19200	TBE-4
	0.19225	TBE-5
	0.19250	TBE-6
Figure 4(d)	γ_2	
	0.000 000 0125	TBE-1
	0.000 000 075	TBE-2
	0.000 000 095	TBE-3
	0.000 000 125	TBE-4
	0.000 000 225	TBE-5
	0.000 000 425	TBE-6
Figure 4(e)	γ_5	
	0.845	TBE-1
	0.850	TBE-2
	0.855	TBE-3
	0.860	TBE-4
	0.865	TBE-5

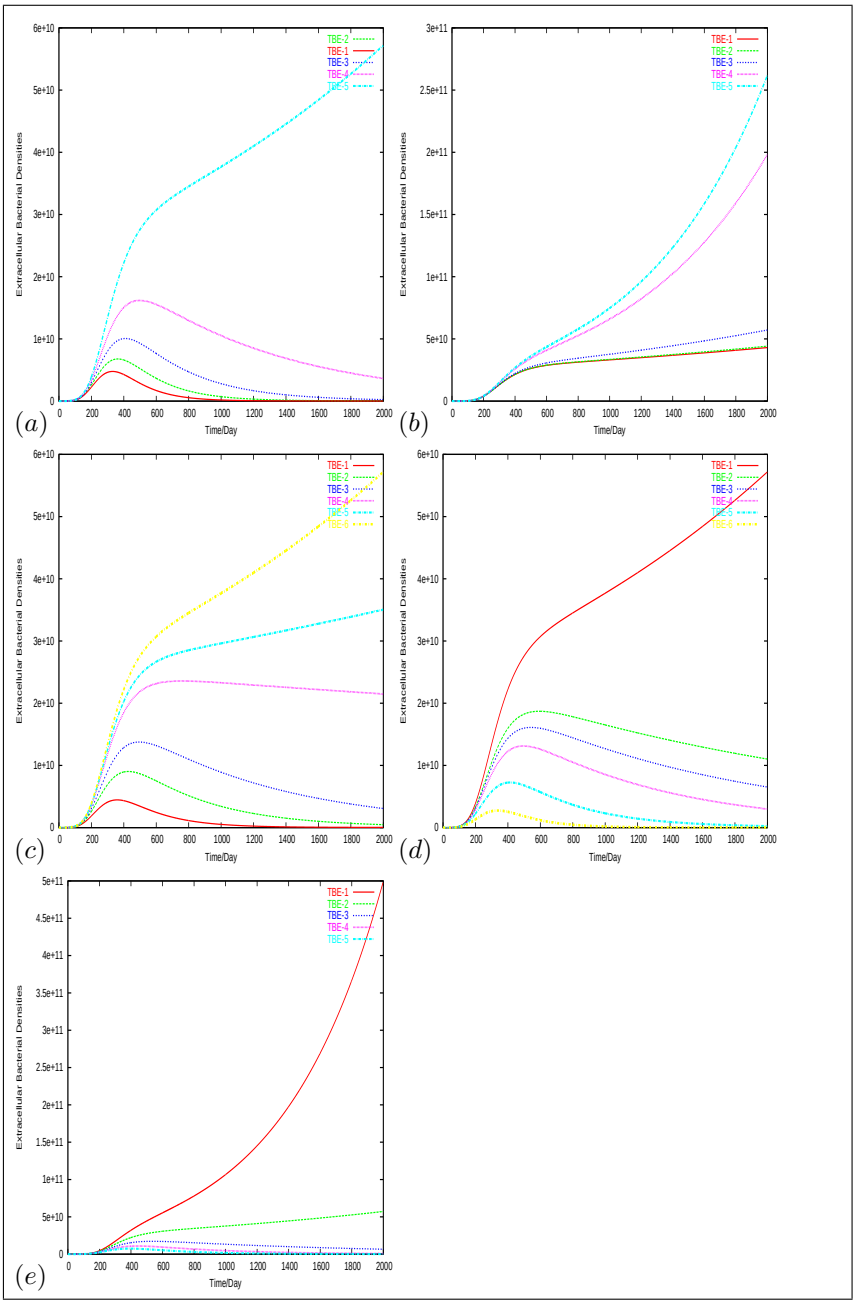

FIGURE 4. Graph of numerical solution showing uncertainty and sensitivity of parameters k_2, k_5, γ_2, γ_4 and γ_5. These parameters contribute to outcome variability when perturbed. Results in this figure were arrived at when conditions that lead to active disease were used but varying only the parameter under investigation. Initial conditions used here are $M_R(0) = 100000$, $T_{BE}(0) = 2000$, $M_I(0) = 0$, $M_A(0) = 0$, $T_{BI}(0) = 0$, $T(0) = 500$ and $C(0) = 140$. Details of parameter variations are given in Table 2, while other fixed parameter values are given in Table 1

6. **Discussion.** In this paper we have presented a mathematical model of the immune response to Mtb infection, which addresses the importance and effects of CTLs in the human immune response during infection with TB. Using the model, we analyzed the effects of each component of the immune system (namely, resting macrophages, activated macrophages, CD4+ helper T cells and CTLs) in determining disease progression. We determined parameters which contribute more and those which play a central role in the progression of the disease. We deduced that active disease is largely attributable to the ability of Mtb pathogen to persist outside the intracellular environment. The ability of Mtb to persist outside macrophages results in fast development or progression to active disease, and the ability of the pathogen to persist inside macrophages gives rise to latent infection. The ability of activated macrophages to kill the Mtb pathogen cannot effectively control the disease, since the Mtb pathogen has mechanisms of escaping destruction inside macrophages. The level of CD8+ T cells has an impact on the control of the disease, since they can directly kill infected macrophages and also kill intracellular and extracellular bacteria. The numerical results show that high levels of CTLs result in latent infection, while low levels result in primary disease. The role of CD4+ helper T cells (combined effects of Th1 and Th2) does not really show effects which influence the control of the disease, but it seems to be supportive to the levels of CTLs. This is in line with the fact that in HIV infection the drop in the CD4+ T cell count results in TB reactivation and fast progression to active disease [19].

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E-mail address: gmagombedze@nust.ac.zw
E-mail address: wgarira@nust.ac.zw
E-mail address: emwenje@nust.ac.zw