MATHEMATICAL ANALYSIS OF MACROPHAGE-BACTERIA INTERACTION IN TUBERCULOSIS INFECTION

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Abstract. Tuberculosis (TB) is a leading cause of death from infectious disease. TB is caused mainly by a bacterium called Mycobacterium tuberculosis which often initiates in the respiratory tract. The interaction of macrophages and T cells plays an important role in the immune response during TB infection. Recent experimental results support that active TB infection may be induced by the dysfunction of Treg cell regulation that provides a balance between anti-TB T cell responses and pathology. To better understand the dynamics of TB infection and Treg cell regulation, we build a mathematical model using a system of differential equations that qualitatively and quantitatively characterizes the dynamics of macrophages, Th1 and Treg cells during TB infection. For sufficiently analyzing the interaction between immune response and bacterial infection, we separate our model into several simple subsystems for further steady state and stability studies. Using this system, we explore the conditions of parameters for three situations, recovery, latent disease and active disease, during TB infection. Our numerical simulations support that Th1 cells and Treg cells play critical roles in TB infection: Th1 cells inhibit the number of infected macrophages to reduce the chance of active disease; Treg cell regulation reduces the immune response to stabilize the dynamics of the system.

1. Introduction. Tuberculosis (TB) is one of the major causes of death by infectious disease. Almost one-third of the world population is under the risk of TB infection. Only 5-10% of TB-infected individuals develop active TB while other infected individuals are able to control infection and reach a stage of latent disease [9]. What distinguishes these different infection outcomes is an active topic of TB infection study.

TB is caused mainly by a bacterium called Mycobacterium tuberculosis (Mtb) which often initiates in the respiratory tract. During TB infection, macrophages play a role as both victims and heroes. Macrophages are infected by Mtb; on the other hand, macrophages take up TB bacteria [3, 16, 22]. Macrophages can become activated under the exposure to bacteria with the catalysis of some cytokines.

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Activated macrophages increase metabolism and an ability to phagocytosis to kill intracellular pathogens [3, 22].

T cell-mediated immune system has to maintain both a state of tolerance toward commensal bacteria and to protect the host from pathogens [17]. Naive CD4+ T cells are activated to Th0 cells at the site of infection and then differentiate into different cell types depending on signals received [4]. An important type of T cell, Th1 cell, can kill infected macrophages but not destroy their resident bacteria. During TB infection, bacteria may either infect macrophages or be killed by activated macrophages; intracellular bacteria can down-regulate apoptosis of their host macrophages to prolong their survival within the protective intracellular environment [4, 14, 31]. Th1 cells can inhibit the infection by reducing the portion of intracellular bacteria. Also, the population of Th1 cell is regulated by several cytokines, including IL-10, IL-12, IFN-γ. IL-10 is produced mainly by macrophages and induces deactivation of macrophages, inhibition of T cell proliferation and suppression of cytokine production. IL-12 is produced by activated and infected macrophages and can induce differentiation to Th1 lymphocytes and enhance the production of IFN-γ which is the key to activate resting macrophages and enhance the differentiation to Th1 cells.

Mathematical modeling provides an important tool to analyze the complex system of TB infection [13, 14, 19, 20, 26, 31]. In [31], an ODE model was proposed to describe the cellular and cytokine control network operational during tuberculosis infection. Three populations of macrophages and bacteria were included in the model and the interactions among Th0, Th1 and Th2 cells and the dynamics of cytokines were discussed as well. Using this model, their computational simulations were used to explore the effects of perturbing different factors in the immune response to TB, including expression of cytokines and recruitment of macrophages and T cell. In [14], an extended mathematical model was based on [31] and it extended the model to a two-compartmental model to capture the important processes of cellular activation and priming that happen between the lung and the nearest draining lymph node. A stochastic model was proposed in [20] to study the interaction of the macrophage and bacterial populations and the results support that stochasticity in the bacterial population or host response could contribute to the diverse incubation periods observed in exposed individuals. However, most of the studies were based on complex mathematical models so the results were usually concluded by computational simulations but not theoretical analysis.

Recent studies suggest that a type of T cell, Treg cell, plays a critical role in TB infection [6, 8, 21, 30]. Treg cells exert an immunosuppressive effect on Th cells and correlate to immune activation throughout the various stages of TB infection: higher bacterial load and T cell response stimulate to a higher level of Treg cells to balance between anti-TB T cell responses and immune-mediated pathology. However, more questions rise as the research on TB infection continues to advance. For example,

- Other than immunosuppressive effect, how does Treg cell play a role on maintaining stable homeostasis?
- Does Treg regulation involve in maintaining latency during TB infection?
- When Treg regulation is overactivated, what kinds of treatment can let the system return back to latency?

Here, we will apply a mathematical model to address these several questions and provide quantitative insights for studying TB infection.
Based on the new studies of the role of Treg cell in TB infection, we build up a mathematical model which consists of the dynamics of macrophages, Th1 and Treg cells, and the change of bacteria concentration during infection. Our model is based on the study of [14, 31] with including Treg regulation and simplification by implicitly modeling the effects of cytokines. For sufficiently understanding the interaction between immune response and bacterial infection, we separate our complex system into several simple subsystems for further steady state and stability studies. In this paper, a complete mathematical description of the model is presented at first in order to have a full picture of the whole system. In the later sections, we follow the order from simple to difficult cases to explore the trend of the bacterial amount under different situations. Using this model system, we explore the conditions of parameters for three situations, recovery, latent disease and active disease, during TB infection. We also apply numerical simulations to discuss the roles of Th1 cells and Treg cells in TB infection: Th1 cells inhibit the number of infected macrophages to reduce the portion of bacteria releasing from infected macrophages; Treg cell regulation reduces the immune response to stabilize the dynamics of the system.

2. Mathematical model.

Notations. The model is based on the schematic diagram of the infection-associated network shown in Figure 1. The model includes six variables represented by the following notations:

\( M_r \): Number of resting macrophages per ml
\( M_i \): Number of infected macrophages per ml
\( M_a \): Number of activated macrophages per ml
\( B \): Number of extracellular bacteria per ml
\( T_1 \): Number of Th1 cells per ml
\( T_r \): Number of Treg cells per ml

Differential equations for macrophages. For resting macrophages, there is a source of new cells coming into the site with a rate \( \alpha_{M_r} \) and a natural death of cells at a rate \( \mu_{M_r} \). In the system, resting macrophages undergo three processes: enhanced recruitment, infection and activation. During TB infection, additional resting macrophages are recruited to the site by infected and activated macrophages at rates \( \beta_{M_r} \) and \( \omega_1 \beta_{M_r} \), respectively [14,24,31]. Resting macrophages may not be able to clear their bacteria load and become infected macrophages at a maximal rate \( k_{MB} \) [7,14]. Meanwhile, resting macrophages may be activated in response to the concentration of bacteria [14] and IFN-\( \gamma \) produced by Th1 cells [3,15,28]; activated macrophages may be deactivated in response to IL-10 produced by Treg cells [15]. Here we assume that cytokines, including IFN-\( \gamma \) and IL-10, have faster dynamics so that we can simplify the process by considering that Th1 cells directly induce macrophage activation with a maximal rate \( \gamma_a \) while Treg cells directly induce deactivation of macrophages with a maximal rate \( \gamma_r \). The differential equation for resting macrophage concentration becomes:

\[
\frac{dM_r}{dt} = \alpha_{M_r} + \beta_{M_r}(M_i + \omega_1 M_a) - k_{MB} M_r \frac{B}{B + K_B} + \gamma_r M_a \frac{T_r}{T_r + Q_r}
\]
$- \frac{\gamma_a M_r}{B + Q_B} \frac{B}{T_1 + Q_1} - \frac{\mu_{M_r}}{\text{death}}.$

Other than natural turnover, infected macrophages may be eliminated through apoptosis by Th1 cell-mediated immunity at a rate $k_{MT}$ [27]. The actual rate of this effect depends on the ratio, $T_1/(M_i + \epsilon)$ where $\epsilon \ll 0$ [14, 31]. Bursting of infected macrophages happens at a rate $\gamma_i$ to increase the concentration of extracellular bacteria [14]. The differential equation for infected macrophage concentration can be formulated as

$$\frac{dM_i}{dt} = \frac{k_{MB} M_r B}{B + K_B} - \frac{k_{MT} M_i T_1/(M_i + \epsilon)}{T_1/(M_i + \epsilon) + K_1} - \frac{\gamma_i M_i}{\text{bursting}} - \frac{\mu_{M_i}}{\text{death}}.$$ 

Similarly, the equation for activated macrophages contains three processes: activation, deactivation and death. The differential equation for activated macrophage concentration is

$$\frac{dM_a}{dt} = \frac{\gamma_a M_r B}{B + Q_B} \frac{T_1}{T_1 + Q_1} - \frac{\gamma_r M_a T_r}{T_r + Q_r} - \frac{\mu_{M_a}}{\text{death}}.$$

**Figure 1. Model diagram of bacteria, T cells and macrophages interaction.** The Sharp arrow means activation; blocked arrow means inhibition. When bacteria encountering resting macrophages, some macrophages will be activated and become activated macrophages ($M_a$), while some will be infected and become infected macrophages ($M_i$). $M_i$ may be deactivated in response to regulatory T cells (Treg). Th1 cells will induce the activation of $M_a$. $M_a$ and $M_r$ will inhibit the growth of bacteria. Bursting of $M_i$ will increase the concentration of bacteria. $M_i$ may be eliminated by Th1 cells. Th1 cells activation will increase in response to $M_i$ and $M_a$, and decrease by Treg cells’ inhibition. Treg cells will be activated by Th1 cells and $M_a$. 
Differential equations for bacteria. Extracellular bacteria grow at a rate $\alpha_B$ and killed by either resting or activated macrophages at rates $k_{BM}$ and $\omega_2 k_{BM}$, respectively [14, 31]. For the exchange of bacteria between extracellular and intracellular compartments can be concluded as two processes: releasing bacteria after infected macrophage bursting and extracellular bacteria becoming intracellular when host macrophages being infected. We assume that $N$ bacteria are released after infected macrophage bursting and a macrophage carries approximately one-half of $N$ [14, 31]. Here we get the formula for the dynamics of $B$:

$$\frac{dB}{dt} = \alpha_B B - k_{BM} (M_i + \omega_2 M_a) B - k_{MB} \frac{N}{2} \frac{B}{B + K_B}$$

Differential equations for T cells. Activated and infected macrophages induce Th1 development through IL-12 [12, 31] while Treg cells provide negative regulatory signals on Th1 cell activation [11, 30]. On the other hand, Treg cell activation is induced by the two types of cytokine, IL-10 and IL-2, which are produced by activated macrophages and Th1 cells, respectively [1, 10]. Similar to the equations for macrophages, we assume that the dynamics of cytokines are much faster than that of cells so that we simplify the processes by considering that Th1 and Treg cell activations are directly increased by the cell concentrations. Overall, we have differential equations for $T_1$ and $T_r$ as:

$$\frac{dT_1}{dt} = \alpha_{T_1} (M_i + \omega_3 M_a) - \frac{1}{1 + T_r/K_r} - \mu_{T_1} T_1,$$

$$\frac{dT_r}{dt} = \alpha_{T_r} M_a + \beta_{T_r} T_1 - \mu_{T_r} T_r.$$
\[ \frac{dT_r}{dt} = \alpha_T M_a + \beta_T T_1 - \mu_T T_r, \quad (6) \]

where all the variables and the parameters are non-negative. Table 1 provides a list of the definitions of the parameters.

In the later sections, our study will start from a simple subsystem (Figure 2) and be extended to the full system systematically to understand the interaction between immune response and bacterial infection.

### Table 1. The definitions of the parameters used in the equations (1)-(6).

| Parameter | Definition |
|-----------|------------|
| \( \alpha_{Mr} \) | \( M_r \) source |
| \( \alpha_B \) | Bacteria growth rate |
| \( \alpha_{T_1} \) | \( T_1 \) activation rate by \( M_i \) and \( M_a \) |
| \( \alpha_{T_r} \) | \( T_r \) activation rate by \( M_a \) |
| \( \beta_{Mr} \) | \( M_r \) recruitment by \( M_i \) and \( M_a \) |
| \( \beta_{T_r} \) | \( T_r \) activation rate by \( T_1 \) |
| \( k_{MB} \) | Infection rate |
| \( k_{MT} \) | \( T_1 \) immunity rate |
| \( k_{BA} \) | Death rate of bacteria by \( M_r \) and \( M_a \) |
| \( \gamma_r \) | Deactivation rate of macrophage |
| \( \gamma_a \) | Activation rate of macrophage |
| \( \gamma_i \) | Bursting rate of \( M_i \) |
| \( \mu_{Mr} \) | Death rate of \( M_r \) |
| \( \mu_{M_i} \) | Death rate of \( M_i \) |
| \( \mu_{M_a} \) | Death rate of \( M_a \) |
| \( \mu_{T_1} \) | Death rate of \( T_1 \) |
| \( \mu_{T_r} \) | Death rate of \( T_r \) |
| \( \omega_1 \) | Ratio in \( M_r \) recruitment |
| \( \omega_2 \) | Ratio in bacteria killing |
| \( \omega_3 \) | Ratio in \( T_1 \) activation |
| \( K_B \) | Half-saturation constant for \( B \) in infection |
| \( Q_B \) | Half-saturation constant for \( B \) in macrophage activation |
| \( K_{T_1} \) | Half-saturation constant for \( T_1 \) in immunity |
| \( K_T \) | Half-saturation constant for \( T_r \) in inhibition |
| \( Q_T \) | Half-saturation constant for \( T_r \) in macrophage deactivation |
| \( Q_{T_1} \) | Half-saturation constant for \( T_1 \) in macrophage activation |
| \( N \) | Estimated number of bacteria per macrophage |
| \( \bar{N} \) | Number of bacteria releasing from macrophage by Th1 cell immunity |

3. Interplay between bacterial infection and macrophage protection: Two-equation model of \( B \) and \( M_r \). First, we consider the dynamics of a subsystem which only involves \( B \) and \( M_r \), as shown in Figure 2A. In this subsystem, the concentrations of extracellular bacteria and resting macrophages have a mutual inhibition relationship. By considering the system with \( M_i = M_a = T_1 = T_r = 0 \), we get a two-equation subsystem for the dynamics of \( B \) and \( M_r \):
Figure 2. Model diagrams of subsystems for analysis. A) Two-equation model. The interaction between bacteria infection and resting macrophages protection. B) Three-equation model. Bacteria infection, macrophages protection with some resting macrophages are infected and become infected macrophages, which will burst and release bacteria.

\[
\begin{align*}
\frac{dB}{dt} &= \alpha_B B - k_{BM} M_r B - k_{MB} \frac{N}{2} M_r \frac{B}{B + K_B}, \\
\frac{dM_r}{dt} &= \alpha_{M_r} - k_{MB} M_r \frac{B}{B + K_B} - \mu_{M_r} M_r.
\end{align*}
\]

where all the parameters are positive.

Now, we study the steady state solutions and their stability of the subsystem. In our analysis, we are only interested in non-negative steady states which are biologically acceptable. For studying steady state solutions of the system (7)-(8), we define two functions for the right hand side of the two-equation system:

\[
\begin{align*}
f_B(B, M_r) &= \alpha_B B - k_{BM} M_r B - k_{MB} \frac{N}{2} M_r \frac{B}{B + K_B}, \\
\hat{f}_M(B, M_r) &= \alpha_{M_r} - k_{MB} M_r \frac{B}{B + K_B} - \mu_{M_r} M_r.
\end{align*}
\]

We start our analysis from a healthy situation. When a human body stays in a healthy situation, the concentration of extracellular bacteria should be at zero level. Therefore, at the steady state, the level of resting macrophage should be \(\alpha_{M_r}/\mu_{M_r}\). This healthy state \((0, \alpha_{M_r}/\mu_{M_r})\) is one of the steady states of the system (7)-(8).

Next, we consider other steady states which have nonzero concentration of extracellular bacteria. Let \((\hat{B}, \hat{M}_r)\) be a solution of \(f_M(\hat{B}, M_r) = f_B(\hat{B}, M_r) = 0\). If \(\hat{B}\) is nonzero, then from \(f_B(\hat{B}, M_r) = 0\), we get

\[
\hat{M}_r = \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/(B + K_B)}.
\]

When we consider \(\hat{M}_r\) in (9) as a function of \(\hat{B}\), the function is a monotonous increasing function. We can evaluate the possible range of \(\hat{M}_r\) by taking limits on both sides:

\[
\lim_{\hat{B} \to 0} \hat{M}_r = \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \leq \hat{M}_r \leq \lim_{\hat{B} \to \infty} \hat{M}_r = \frac{\alpha_B}{k_{BM}}.
\]
On the other hand, from \( f_M(\hat{B}, \hat{M}_r) = 0 \), we obtain
\[
\hat{M}_r = \frac{\alpha_{M_r}}{k_{MB} \frac{B}{B+K_B} + \mu_{M_r}}.
\]  
(11)

Similar to the previous case, when we consider \( \hat{M}_r \) in (11) as a function of \( \hat{B} \), the function is a monotonous decreasing function. By taking limits on both sides, we can get the possible range of \( \hat{M}_r \):
\[
\lim_{\hat{B} \to \infty} \hat{M}_r = \frac{\alpha_{M_r} \mu_{M_r}}{k_{MB} + k_{MB}(N/2) / K_B} \leq \hat{M}_r \leq \lim_{\hat{B} \to 0} \hat{M}_r = \frac{\alpha_{M_r}}{k_{MB} \mu_{M_r}}.
\]  
(12)

If \( \frac{\alpha_{M_r}}{\mu_{M_r}} < \frac{\alpha_B}{k_{BM} + k_{MB}(N/2) / K_B} \) or \( \frac{\alpha_{M_r}}{\mu_{M_r}} > \frac{\alpha_B}{k_{BM}} \), then the above two possible ranges (10) and (12) will have no intersection with nonzero \( \hat{B} \), which means that there is no steady state solution with nonzero concentration of extracellular bacteria and \((0, \frac{\alpha_{M_r}}{\mu_{M_r}})\) is the only steady state for the system.

Now we first obtain the Jacobian matrix for local stability analysis, then the stability analysis will be investigated in the following three cases:

**Case 1:** \( \frac{\alpha_{M_r}}{\mu_{M_r}} < \frac{k_B + k_{MB}(N/2) / K_B}{\alpha_{M_r}} \)

**Case 2:** \( \frac{\alpha_{M_r}}{\mu_{M_r}} > \frac{k_B}{\alpha_{M_r}} \) and \( \frac{\alpha_{M_r}}{\mu_{M_r}} < \frac{\alpha_B}{k_{BM}} \)

**Jacobi matrix for the two-equation system.** For the local stability analysis, we have to obtain the Jacobian matrix at \((\hat{B}, \hat{M}_r)\) for the two-equation system.

First, we obtain the following derivatives:
\[
\frac{\partial f_B}{\partial B} = \alpha_B - k_{BM} \hat{M}_r - k_{MB} \frac{N}{2} \hat{M}_r \frac{K_B}{(B + K_B)^2},
\]
\[
\frac{\partial f_B}{\partial M_r} = -k_{BM} \hat{B} - k_{MB} \frac{N}{2} \frac{\hat{B}}{B + K_B},
\]
\[
\frac{\partial f_M}{\partial B} = -k_{MB} \hat{M}_r \frac{K_B}{(B + K_B)^2},
\]
\[
\frac{\partial f_M}{\partial M_r} = -k_{MB} \frac{\hat{B}}{B + K_B} - \mu_{M_r}.
\]

When \((\hat{B}, \hat{M}_r) = (0, \frac{\alpha_{M_r}}{\mu_{M_r}})\), we get the Jacobian matrix
\[
J(0, \frac{\alpha_{M_r}}{\mu_{M_r}}) = \begin{pmatrix}
\alpha_B - k_{BM} \frac{\alpha_{M_r}}{\mu_{M_r}} & -k_{MB} \frac{N}{2} \frac{\alpha_{M_r}}{\mu_{M_r}} \frac{1}{K_B} & 0 \\
-k_{MB} \frac{\alpha_{M_r}}{\mu_{M_r}} \frac{1}{K_B} & -\mu_{M_r}
\end{pmatrix}.
\]

The eigenvalues of the Jacobian matrix \(J(0, \frac{\alpha_{M_r}}{\mu_{M_r}})\) can be expressed in
\[
\lambda_1 = -\mu_{M_r} < 0,
\]
\[
\lambda_2 = \alpha_B - k_{BM} \frac{\alpha_{M_r}}{\mu_{M_r}} - k_{MB} \frac{N}{2} \frac{\alpha_{M_r}}{\mu_{M_r}} \frac{1}{K_B}.
\]

**Case 1:** \( \frac{\alpha_{M_r}}{\mu_{M_r}} < \frac{\alpha_B}{k_{BM} + k_{MB}(N/2) / K_B} \)

One unstable steady state
If $\frac{\alpha_{Mr}}{\mu_{Mr}} < \frac{\alpha_B}{k_{BM}+k_{MB}(N/2)/K_B}$, then $\alpha_B > k_{BM} \frac{\alpha_{Mr}}{\mu_{Mr}} + k_{MB} \frac{N \alpha_{Mr}}{2 \mu_{Mr}} \frac{1}{K_B}$. In this case, $\lambda_2$ is a positive number so the steady state solution $(0, \alpha_{Mr}/\mu_{Mr})$ is locally unstable.

Figure 3A is the phase plane portrait when the unstable steady state condition is satisfied. The dotted lines represent the relationship between extracellular bacteria and resting macrophages when $dB/dt = f = 0$ is satisfied, while solid line stands for the amount relationship when $dM_r/dt = g = 0$ is satisfied. The star symbol indicates the only steady state solution $(0, \alpha_{Mr}/\mu_{Mr})$. The phase plane portrait demonstrates that initial TB infection may lead to an active disease in this case. This observation can be verified by the following theorem.

**Theorem 1.** Assume that $\frac{\alpha_{Mr}}{\mu_{Mr}} < \frac{\alpha_B}{k_{BM}+k_{MB}(N/2)/K_B}$. If $B(0) > 0$ and $M_r(0) \geq 0$, then the value of $B(t)$ for the system (7)-(8) will go to infinity when $t$ tends to infinity.

The theorem suggests that when $\frac{\alpha_{Mr}}{\mu_{Mr}} < \frac{\alpha_B}{k_{BM}+k_{MB}(N/2)/K_B}$, a little increase on bacterial concentration at the healthy state $(0, \alpha_{Mr}/\mu_{Mr})$ will lead to active disease with continuous bacterial growth. The detailed proof of Theorem 1 is presented in Appendix A.1.

**Case 2:** $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} > \frac{\alpha_B}{k_{BM}}$

One stable steady state

If $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} > \frac{\alpha_B}{k_{BM}}$, then $\alpha_B < k_{BM} \frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} < k_{BM} \frac{\alpha_{Mr}}{\mu_{Mr}} < k_{BM} \frac{\alpha_{Mr}}{\mu_{Mr}} + k_{MB} \frac{N \alpha_{Mr}}{2 \mu_{Mr}} \frac{1}{K_B}$. In this case, both $\lambda_1$ and $\lambda_2$ are negative numbers. So the equilibrium point $(0, \alpha_{Mr}/\mu_{Mr})$ is a locally stable steady state.

Figure 3B is the phase plane portrait for this case. Similar to Case 1, the phase plane portrait provides us an insight for Theorem 2 and the detailed proof of Theorem 2 is presented in Appendix A.2.

**Theorem 2.** Assume that $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} > \frac{\alpha_B}{k_{BM}}$. The solution of $(B(t), M_r(t))$ for the system (7)-(8) with non-negative initial values will go to the unique steady state solution $(0, \alpha_{Mr}/\mu_{Mr})$ when $t$ tends to infinity. That means the steady state $(0, \alpha_{Mr}/\mu_{Mr})$ is globally stable.

Theorem 2 suggests that, under the condition $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} > \frac{\alpha_B}{k_{BM}}$, the infected individual will return back to the healthy state $B = 0$ after a recovery period.

**Case 3:** $\frac{\alpha_{Mr}}{\mu_{Mr}} > \frac{\alpha_B}{k_{BM}+k_{MB}(N/2)/K_B}$ and $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} < \frac{\alpha_B}{k_{BM}}$

One stable steady state and one unstable steady state

If $\frac{\alpha_{Mr}}{\mu_{Mr}} > \frac{\alpha_B}{k_{BM}+k_{MB}(N/2)/K_B}$ and $\frac{\alpha_{Mr}}{\mu_{Mr}+k_{MB}} < \frac{\alpha_B}{k_{BM}}$, the equilibrium $(0, \alpha_{Mr}/\mu_{Mr})$ is a stable steady state solution. At the same time, the system has another steady state by solving $f_B(B^*, M_r^*) = f_M(B, M_r) = 0$:

$$B^* = \frac{\alpha_{Mr}k_{BM}K_B + \alpha_{Mr}k_{MB}(N/2) - \alpha_B M_r K_B}{\alpha_B k_{MB} + \alpha_B M_r - \alpha_{Mr} K_B},$$

$$M_r^* = \frac{\alpha_{Mr}(N/2) + \alpha_B K_B}{k_{BM} K_B + k_{MB}(N/2) + \mu_{Mr}(N/2)}.$$
A phase plane portraits for the analysis of the two-equation system (7)-(8). A) Case 1 B) Case 2 C) Case 3.

Star symbols indicate the steady states; red dotted lines represent $dM_r/dt = 0$; blue solid lines represent $dB/dt = 0$.

We substitute $(B^*, M_r^*)$ into the Jacobian matrix, and get the determinant of the matrix

$$\det(J(B^*, M_r^*)) = - \left( k_{MB} \frac{B^*}{B^* + K_B} + \mu_{M_r} \right) \left( k_{MB} \frac{N}{2} M_r^* \frac{B^*}{(B^* + K_B)^2} \right) - \left( k_{BM} B^* + k_{MB} \frac{N}{2} \frac{B^*}{B^* + K_B} \right) \left( k_{MB} M_r^* \frac{K_B}{(B^* + K_B)^2} \right) < 0$$

It implies one of the eigenvalues has to be a positive real number and the steady state $(B^*, M_r^*)$ is locally unstable.

Figure 3C is the phase plane portrait for this case which have two steady state solutions $(0, \alpha M_r / \mu_{M_r})$ and $(B^*, M_r^*)$ marked by star symbol in the figure. The following theorem shows how the long-term behavior of bacterial concentration depends on the initial condition. The detailed proof of Theorem 3 is presented in Appendix A.3.

**Theorem 3.** Assume that $\frac{\alpha_{M_r}}{\mu_{M_r}} > \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B}$ and $\frac{\alpha_{M_r}}{\mu_{M_r} + k_{MB}} < \frac{\alpha_B}{k_{BM}}$.

1. If $0 \leq B(0) < B^*$ and $f_B(B(0), M_r(0)) < 0$ (Area 1 in Figure 3C), then the value of $B(t)$ for the system (7)-(8) will go to the steady state $(0, \alpha M_r / \mu_{M_r})$ when $t$ tends to infinity;
2. If \( B(0) > B^* \) and \( f_B(B(0), M_r(0)) > 0 \) (Area 2 in Figure 3C), then the value of \( B(t) \) for the system (7)-(8) will go to infinity when \( t \) tends to infinity.

Theorem 3 suggests that, under the condition of Case 3, the infected individual will return back to the healthy state if the bacterial concentration is less than certain threshold \( B^* \) and the macrophage concentration is high enough \( (f_B(B(0), M_r(0)) < 0) \); active disease will happen if the bacterial concentration is larger than certain threshold \( B^* \) and the macrophage concentration is sufficiently low \( (f_B(B(0), M_r(0)) > 0) \).

From the previous analysis, we find that there are only two disease situations, recovery and active disease. Latent TB infection (non-zero finite bacterial concentration) is not observed in the two-equation model. In the next section, after we include infected macrophages, \( M_i \), into the model, we will find that there is a stable steady state with non-zero bacterial concentration, corresponding to latent disease.

4. Effect of infected macrophage: Three-equation model of \( B, M_r \) and \( M_i \).

Since the resting macrophages may not be able to clear their bacteria load, they will become infected macrophages which may play an important role in enhancing the growth of bacterial population. Here we consider the dynamics of a subsystem which involves three variables \( B, M_r \) and \( M_i \), as shown in Figure 2B. By considering the full system (1)-(6) with \( M_a = \bar{M}_a = T_l \), we get a three-equation subsystem for the dynamics of \( B, M_r \) and \( M_i \):

\[
\frac{dB}{dt} = \alpha_B B - k_{BM} M_r B - k_{MB} N M_r \frac{B}{2 M_r + B + K_B} + \gamma_i N M_i, \quad (13)
\]

\[
\frac{dM_r}{dt} = \alpha_{M_r} + \beta M_i - k_{MB} M_r \frac{B}{B + K_B} - \mu_{M_r} M_r, \quad (14)
\]

\[
\frac{dM_i}{dt} = k_{MB} M_r \frac{B}{B + K_B} - \gamma_i M_i - \mu_{M_i} M_i, \quad (15)
\]

where all the parameters are positive.

Let \((\hat{B}, \hat{M}_r, \hat{M}_i)\) be the steady state solution for the three-equation system. For steady-state study, we consider the steady state system by setting \( dM_r/dt = dM_i/dt = dB/dt = 0 \) to be zero:

\[
0 = \alpha_B \hat{B} - k_{BM} \hat{M}_r \hat{B} - k_{MB} N \frac{\hat{M}_r}{2} \frac{\hat{B}}{B + K_B} + \gamma_i N \hat{M}_i, \quad (16)
\]

\[
0 = \alpha_{M_r} + \beta M_i - k_{MB} \hat{M}_r \frac{\hat{B}}{B + K_B} - \mu_{M_r} \hat{M}_r, \quad (17)
\]

\[
0 = k_{MB} \hat{M}_r \frac{\hat{B}}{B + K_B} - \gamma_i \hat{M}_i - \mu_{M_i} \hat{M}_i. \quad (18)
\]

By (18), \( \hat{M}_i \) can be expressed as

\[
\hat{M}_i = \frac{k_{MB}}{\gamma_i + \mu_{M_i}} \hat{M}_r \frac{\hat{B}}{B + K_B}. \quad (19)
\]

Substitute (19) into (16) and (17), we have two equations for \( \hat{M}_r \) and \( \hat{B} \):

\[
0 = \alpha_B \hat{B} - k_{BM} \hat{M}_r \hat{B} - k_{MB} N \frac{\hat{M}_r}{2} \frac{\hat{B}}{B + K_B} = g_B(\hat{B}, \hat{M}_r), \quad (20)
\]
where $k_{MMB} = k_{MB} \left(1 - \frac{3\nu}{\gamma_i + \mu_{M_i}}\right)$ and $k_{MBB} = k_{MB} \left(1 - \frac{2\nu}{\gamma_i + \mu_{M_i}}\right)$. It is easy to check that $(\hat{M}, \hat{M}_r, \hat{M}_i) = (0, \alpha_{M_r}/\mu_{M_r}, 0)$ is one of the steady state solutions which satisfy (16)-(18).

For further analyzing for the existence of another steady state, we define four useful terms $L_M$, $R_M$, $L_B$ and $R_B$:

$$L_M = \frac{\alpha_{M_r}}{\mu_{M_r}}, \quad R_M = \frac{\alpha_{M_r}}{\mu_{M_r} + k_{MMB}};$$

$$L_B = \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B}, \quad R_B = \frac{\alpha_B}{k_{BM}}.$$

Note that $L_B$ and $L_M$ represent the solutions of $\hat{M}_r$ from the equations $\frac{g_M(\hat{B}, \hat{M}_r)}{\hat{B}} = 0$ and $g_M(\hat{B}, \hat{M}_r) = 0$ when $\hat{B} \to 0$, respectively; $R_B$ and $R_M$ represent the solutions of $\hat{M}_r$ from the equations $g_B(\hat{B}, \hat{M}_r) = 0$ and $g_M(\hat{B}, \hat{M}_r) = 0$ when $\hat{B} \to \infty$, respectively. In the biological implications, $L_M < L_B$ ($L_M > L_B$) corresponds to weak (strong) macrophage regulation when bacterial concentration is low; $R_M < R_B$ ($R_M > R_B$) corresponds to weak (strong) macrophage regulation when bacterial concentration is high.

If $k_{MMB}$ and $k_{MBB}$ are both positive, the two equations (20) and (21) have same forms with $f_M = 0$ by replacing $k_{MB}$ by $k_{MMB}$ and $f_B = 0$ by replacing $k_{MB}$ by $k_{MBB}$ so we can apply same way used in the two-equation system to study the number of steady states. For other cases, we can use similar method to consider the intersection points between two curves (20) and (21) to determine the number of steady states. When two steady states exist, the steady state with non-zero bacterial concentration can be expressed as

$$B^* = \frac{\alpha_M k_{BM} K_B + \alpha_M k_{MBB}(N/2) - \alpha_B \mu_M K_B}{\alpha_B k_{MMB} + \alpha_B \mu_{M_r} - \alpha_M k_{BM}} = K_B \frac{1/L_B - 1/L_M}{1/R_M - 1/R_B},$$

$$M_r^* = \frac{\alpha_M (N/2) + \alpha_B K_B}{k_{BM} K_B + k_{MBB}(N/2) + \mu_M (N/2)},$$

$$M_i^* = \frac{k_{MB} M^*}{\gamma_i + \mu_{M_i}} \frac{B^* + K_B}{B^* + K_B} = \frac{1}{\gamma_i + \mu_{M_i} - \beta_{M_i}} \frac{\alpha_M k_{BM} K_B + \alpha_M k_{MBB}(N/2) - \alpha_B \mu_M K_B}{k_{BM} K_B + k_{MBB}(N/2) + \mu_M (N/2)}.$$

By considering $M_r^* \geq 0$, $M_i^* \geq 0$ and $B^* > 0$, the expression above also provides a way to obtain the condition for the existence of the steady state with non-zero bacterial concentration. The results are summarized in Table 2.

**Jacobian matrix for the three-equation system.** For the local stability analysis, we calculate the Jacobian matrix at a steady state solution $(\hat{B}, \hat{M}_r, \hat{M}_i)$ for the three-equation system (13)-(15). When $(\hat{B}, \hat{M}_r, \hat{M}_i) = (0, \alpha_{M_r}/\mu_{M_r}, 0)$, we get:

$$J(0, \alpha_{M_r}/\mu_{M_r}, 0) = \begin{pmatrix}
\alpha_B - k_{MMB} \frac{\alpha_M}{\mu_{M_r}} - k_{MB} \frac{N}{2} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & \alpha_B - k_{MMB} \frac{\alpha_M}{\mu_{M_r}} - k_{MB} \frac{N}{2} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & \gamma_i N \\
-k_{MB} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & -k_{MB} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & -\mu_{M_r} \frac{\beta_{M_i}}{\beta_{M_i} - \gamma_i} \\
k_{MB} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & k_{MB} \frac{\alpha_M}{\mu_{M_r}} \frac{1}{K_B} & 0
\end{pmatrix}. $$
The eigenvalues can be obtained by solving the characteristic equation \( \det(J(0, \alpha_M/\mu_M, 0) - \lambda I) = 0 \):

\[
(-\mu_M - \lambda) \left[ \left( \alpha_B - k_{BM}\frac{\alpha_M}{\mu_M} - k_{MB}\frac{N\alpha_M}{2\mu_MK_B} \right) \left( \gamma_i - \mu_M - \lambda \right) \right. \\
\left. - \left( \gamma_i Nk_{MB}\frac{\alpha_M}{\mu_MK_B} \right) \right] = 0. 
\] (22)

All eigenvalues of the Jacobian matrix \( J \) have negative real parts if and only if

\[- \left( \alpha_B - k_{BM}\frac{\alpha_M}{\mu_M} - k_{MB}\frac{N\alpha_M}{2\mu_MK_B} \right) \left( \gamma_i + \mu_M \right) - \left( \gamma_i Nk_{MB}\frac{\alpha_M}{\mu_MK_B} \right) > 0, \]

which can be expressed as

\[ (L_M - L_B)/L_B > 0, \]

that provides a stability condition for the steady state solution \( (0, \alpha_M/\mu_M, 0) \).

### Table 2. Steady state analysis for the three-equation system (13)-(15).

| Steady states | Status |
|---------------|--------|
| \( L_M < L_B \) | Latent disease |
| \( R_M < R_B \) | Stable \((B^*, M^*_r, M^*_t)\) |
| If \( R_M < 0 \), Two steady states | Active disease |
| Unstable steady state \((0, \alpha_M/\mu_M, 0)\) | (Unstable \((B^*, M^*_r, M^*_t)\)) |
| Stable or unstable steady state \((B^*, M^*_r, M^*_t)\) | |
| If \( R_M > 0 \), One steady state | |
| Unstable steady state \((0, \alpha_M/\mu_M, 0)\) | |

| \( L_M < L_B \) | Latent disease |
| \( R_M > R_B \) | Stable \((B^*, M^*_r, M^*_t)\) |
| Two steady states | Active disease |
| Unstable steady state \((0, \alpha_M/\mu_M, 0)\) | (Unstable \((B^*, M^*_r, M^*_t)\)) |
| Stable or unstable steady state \((B^*, M^*_r, M^*_t)\) | |

| \( L_M > L_B \) | Active disease or recovery |
| \( R_M < R_B \) | (depends on initial values) |
| If \( R_M > 0 \) and \( L_B > 0 \), Two steady states | |
| Stable steady state \((0, \alpha_M/\mu_M, 0)\) | |
| Unstable steady state \((B^*, M^*_r, M^*_t)\) | |
| If \( R_M < 0 \) and \( R_B/L_B > R_M/L_M \), | |
| Two steady states | |
| Unstable steady state \((0, \alpha_M/\mu_M, 0)\) | |
| Stable steady state \((B^*, M^*_r, M^*_t)\) | |
| If \( R_M < 0 \) and \( L_B > 0 \), One steady state | |
| Stable steady state \((0, \alpha_M/\mu_M, 0)\) | |

| \( L_M > L_B \) | Recovery |
| \( R_M > R_B \) | |
| If \( L_B < 0 \), Two steady states | |
| Unstable steady state \((0, \alpha_M/\mu_M, 0)\) | |
| Stable steady state \((B^*, M^*_r, M^*_t)\) | |
| If \( L_B > 0 \), One steady state | |
| Stable steady state \((0, \alpha_M/\mu_M, 0)\) | |

For the conditions, \( L_M < L_B \) \((L_M > L_B)\) corresponds to weak (strong) macrophage regulation when bacterial concentration is low; \( R_M < R_B \) \((R_M > R_B)\) corresponds to weak (strong) macrophage regulation when bacterial concentration is high.
For another steady state solution \((B^*, M_r^*, M_b^*)\) with non-zero bacterial concentration, the characteristic equation \(\det(J(B^*, M_r^*, M_b^*) - \lambda I) = 0\) is obtained as

\[-\lambda^3 + \Omega_2 \lambda^2 + \Omega_1 \lambda + \Omega_0 = 0,\]

where

\[
\begin{align*}
\Omega_2 &= k_{MBB} \frac{N}{2} M_r^* \frac{B^*}{B^* + K_B} - \frac{1}{2} M_r^* \frac{K_B}{(B^* + K_B)^2} - k_{MBB} \frac{B^*}{B^* + K_B} - \mu_M - \gamma_i - \mu_M, \\
\Omega_1 &= -\left(\gamma_i + \mu_M\right) \frac{\alpha_r}{M_r^*} - \mu_M k_{MBB} N \frac{M_r^*}{2} \frac{K_B}{(B^* + K_B)^2} + \frac{k_{MBB} B^* M_r^* K_B \alpha_B}{(B^* + K_B)^2} L_B + \mu_M k_{M} N \frac{M_r^*}{2} \frac{B^*}{(B^* + K_B)^2} , \\
\Omega_0 &= \frac{B^* M_r^* K_B \alpha_r \alpha_B (\gamma_i + \mu_M)}{(B^* + K_B)^2} \left( \frac{1}{R_MB} - \frac{1}{L_MB} \right).
\end{align*}
\]

By the fact that \(L_B < 0\) implies \(k_{MBB} < 0\), it is easy to check that if \(L_B < 0\), every term in \(\Omega_1\) and \(\Omega_2\) is negative so \(\Omega_1\) and \(\Omega_2\) are negative. Also, if \(L_B < 0\), we have

\[-\Omega_2 > \mu_M - \gamma_i + \mu_M_i > 0,\]

and

\[-\Omega_1 > -\left(\gamma_i + \mu_M\right) k_{MBB} N \frac{M_r^*}{2} \frac{B^*}{(B^* + K_B)^2} - \frac{k_{MBB} B^* M_r^* K_B \alpha_B}{(B^* + K_B)^2} L_B > 0;\]

hence,

\[
\begin{align*}
\Omega_1 \Omega_2 &> -\mu_M, (\gamma_i + \mu_M_i) k_{MBB} N \frac{M_r^*}{2} \frac{B^*}{(B^* + K_B)^2} \\
&- \left(\gamma_i + \mu_M\right) \frac{k_{MBB} B^* M_r^* K_B \alpha_B}{(B^* + K_B)^2} L_B \\
&\Omega_1 \Omega_2 > \left(\gamma_i + \mu_M\right) B^* M_r^* K_B \frac{N}{2} \frac{1}{K_B} - \frac{k_{MBB} \alpha_B}{L_B} > 0.
\end{align*}
\]

By the fact that \(k_{MB} > k_{M} MB\) and the definitions of \(L_B, L_M, R_B\) and \(R_M\), we have if \(L_B < 0\), then

\[
\begin{align*}
\Omega_1 \Omega_2 &> \frac{\left(\gamma_i + \mu_M\right) B^* M_r^* K_B}{(B^* + K_B)^2} \left( \frac{-\mu_M, k_{MBB} N}{2} \frac{1}{K_B} - \frac{k_{MBB} \alpha_B}{L_B} \right) \\
\Omega_1 \Omega_2 &> \frac{\left(\gamma_i + \mu_M\right) B^* M_r^* K_B}{(B^* + K_B)^2} \left( \frac{-\mu_M, \alpha_B}{L_B} - \frac{1}{R_B} \right) \frac{k_{MBB} \alpha_B}{L_B} \left( \mu_M + k_{M} MB \right) \frac{1}{L_B} - \frac{\mu_M, R_M}{R_B} \\
\Omega_1 \Omega_2 &> \frac{\left(\gamma_i + \mu_M\right) B^* M_r^* K_B \alpha_B \alpha_M}{(B^* + K_B)^2} \left( \frac{1}{R_MB} - \frac{1}{L_MB} \right) = -\Omega_0.
\end{align*}
\]

From the analysis above, several stability conditions can be obtained and listed as the following:

1. If \(L_M > L_B > 0\) and \(R_B > R_M > 0\), then \(\Omega_0\) is positive and at least one of eigenvalues has a positive real part. Under this condition, \((B^*, M_r^*, M_b^*)\) is locally unstable;
2. If \(L_M > 0 > L_B, R_B > 0 > R_M\) and \(\frac{R_B}{L_B} > \frac{R_M}{L_M}\), then \(\Omega_2, \Omega_1\) and \(\Omega_0\) are both negative and \(\Omega_1 \Omega_2 > -\Omega_0\). Through Routh-Hurwitz criterion [5], all eigenvalues have negative real parts and \((B^*, M_r^*, M_b^*)\) is locally stable;
3. If $L_M > 0 > L_B$ and $R_M > R_B > 0$, then $\Omega_2$, $\Omega_1$ and $\Omega_0$ are both negative and $\Omega_1 \Omega_2 > -\Omega_0$. Through Routh-Hurwitz criterion, all eigenvalues have negative real parts and $(B^*, M_r^*, M_t^*)$ is locally stable.

4. For the remaining two cases ($L_M < L_B$ and $R_B > 0 > R_M$ and $(L_M < L_B$ and $R_M > R_B)$, $(B^*, M_r^*, M_t^*)$ may be stable or unstable. In these two cases, $\Omega_1$ can be negative or positive. For example, we set $\alpha_{Mb} = \alpha_B = \mu_B = k_{MB} = K_B = 1 \text{ day}^{-1}$, $k_{RB} = 1.01 \text{ day}^{-1}$ and $N = 7$, we have

- when $\mu_{M_L} = 10^{-4} \text{ day}^{-1}$, $\beta_{M_r} = 1.994 \times 10^{-4} \text{ day}^{-1}$ and $\gamma_i = 1.01 \times 10^{-4} \text{ day}^{-1}$, the conditions $L_M < L_B$ and $R_M > R_B$ are satisfied, $\Omega_1 > 0$ and $(B^*, M_r^*, M_t^*)$ is locally unstable;
- when $\mu_{M_L} = 10^{-2} \text{ day}^{-1}$, $\beta_{M_r} = 1.994 \times 10^{-2} \text{ day}^{-1}$ and $\gamma_i = 1.01 \times 10^{-2} \text{ day}^{-1}$, the conditions $L_M < L_B$ and $R_M > R_B$ are satisfied, $\Omega_1 < 0$ and $(B^*, M_r^*, M_t^*)$ is locally stable.

The stability analysis is summarized in Table 2. Although the local stability analysis is valid only for the case when the initial condition is close to the steady state, the global behavior can be tested through numerical simulations. We test the global behavior of the system with initial conditions, $B(0) = 100$, $M_r(0) = \alpha_{M_r}/\mu_{M_r}$ and $M_t(0) = 0$ and 100 random sets of parameters from the range estimated from the references and listed in Table 3. All the results are consistent with our stability analysis. Figure 4 shows the simulations for a case that the stable steady state solution $(B^*, M_r^*, M_t^*)$ exists. The steady state of $B$ is changing with different values $\beta_{M_r}$ and $B(t)$ approaches its steady state at the end.

Through the analysis, we obtain the conditions of parameters for achieving different disease statuses. Among all parameters, $\beta_{M_r}$ and $\gamma_i$ are the important parameters for controlling the signs and the values of $L_B$ and $R_M$. Here, we apply the conditions obtained from the local stability analysis to study what disease status will be reached with different combinations of $\beta_{M_r}$ and $\gamma_i$.

Instead of selecting one set of parameters for the model, we study a large number ($10^7$) of sets of parameters randomly chosen in the ranges listed in Table 3. When we set $\beta_{M_r}$ value between 0.01 day$^{-1}$ and 10 day$^{-1}$, and $\gamma_i = 0.04$ day$^{-1}$, almost 99% cases go to the situation that $R_B > R_M > 0$ and $L_M > 0 > L_B$, corresponding to the active disease status; when we reduce $\gamma_i$ to be 0.01 day$^{-1}$ with $\beta_{M_r} \geq 0.04$ day$^{-1}$, almost 99% cases go to the situation that $R_B > 0 > R_M$ and $L_B > L_M$ with a stable steady state $(B^*, M_r^*, M_t^*)$, corresponding to the latent disease status. Comparing to the value of $M_r$, the values of $B^*$ and $M_t^*$ are relatively large when $\beta_{M_r} = 0.04$ day$^{-1}$ (the mean values of $B^*$, $M_r^*$ and $M_t^*$ are $1.71 \times 10^3$, $1.11 \times 10^7$ and $2.23 \times 10^7$, respectively. The results in Figure 4 show that if the value of $\beta_{M_r}$ is increasing from 0.1 to 10 and $\gamma_i$ is kept to be 0.01 day$^{-1}$, the status is kept to be the latent disease and the mean values of $B^*$ and $M_t^*$ are significantly reduced to be 331.89 and $2.21 \times 10^4$, respectively.

5. Activated macrophage and T cell regulation. Through the stability analysis in the previous section, we observe that increasing the values of $\beta_{M_r}$ and decreasing the values of $\gamma_i$ together can inhibit the bacterial growth to change active disease to be latent. In the full model (1)-(6), activated macrophages may enhance the macrophage recruitment, that acts a role in increasing the value of $\beta_{M_r}$; Th1 cells may inhibit the number of infected macrophages to reduce the portion of bacteria releasing from infected macrophages, that acts a role in decreasing the values of $\gamma_i$. However, Th1 cell overactivation will lead to an unbalanced environment, thus reactivating the latent disease and making the disease status unstable.

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Table 3. Parameters used in the equations (1)-(6).

| Parameter | Range for stability tests in Section 4 | Values for simulations in Figs. 4-6 and Figs. 8-10 | Range for OJ tests in Fig. 7 and Fig. 10 | Unit | Reference |
|-----------|--------------------------------------|----------------------------------------------------|------------------------------------------|------|-----------|
| $\alpha_M$ | $3.3 \times 10^0 - 1 \times 10^1$ | $5 \times 10^0$ | $3.3 \times 10^0 - 1 \times 10^1$ | ml$^{-1}$ day$^{-1}$ | [2,31] |
| $\alpha_B$ | $0.01 - 0.1$ | $5 \times 10^{-2}$ | $0.025 - 0.075$ | day$^{-1}$ | [14,31] |
| $\alpha_T$ | - | $5.2 \times 10^{-2}$ | $0.026 - 0.078$ | day$^{-1}$ | [14] |
| $\beta_M$ | $10^2 - 10$ | $1 \times 10^{-2}$ | $10^{-2} - 0.1$ | day$^{-1}$ | [14,31] |
| $\beta_T$ | - | $1 \times 10^{-1}$ | 1 | day$^{-1}$ | Estimated |
| $k_{MB}$ | $0.2 - 0.5$ | $5 \times 10^{-4}$ | $0.2 - 0.5$ | day$^{-1}$ | [14] |
| $k_{MT}$ | - | $1 \times 10^{-4}$ | $0.08 - 0.12$ | day$^{-1}$ | Estimated |
| $k_{BM}$ | $1.25 \times 10^{-3}$ | $2 \times 10^{-5}$ | $1.25 \times 10^{-3} - 2 \times 10^{-5}$ | ml day$^{-1}$ | [3,14] |
| $\gamma_T$ | - | $2 \times 10^{-1}$ | $0.1 - 0.5$ | day$^{-1}$ | Estimated |
| $\gamma_a$ | - | $4 \times 10^{-1}$ | $0.2 - 0.6$ | day$^{-1}$ | Estimated |
| $\beta_i$ | $0.01 - 0.1$ | $4 \times 10^{-2}$ | $0.2 - 0.4$ | day$^{-1}$ | Estimated |
| $\mu_M$ | $1 \times 10^0$ | $1 \times 10^{-2}$ | $1 \times 10^{-2}$ | day$^{-1}$ | [14,29,31] |
| $\mu_T$ | - | $1 \times 10^{-2}$ | $1 \times 10^{-2}$ | day$^{-1}$ | [14,29,31] |
| $\omega_1$ | - | $3.33 \times 10^{-1}$ | $3.33 \times 10^{-1}$ | day$^{-1}$ | [14,31] |
| $\omega_2$ | - | $3.33 \times 10^{-1}$ | $3.33 \times 10^{-1}$ | day$^{-1}$ | [14,31] |
| $\omega_3$ | - | $7.14$ | $7.14$ | day$^{-1}$ | [14] |
| $K_B$ | $1 \times 10^1$ | $1 \times 10^1$ | $1 \times 10^1$ | ml$^{-1}$ | [31] |
| $Q_B$ | - | $1 \times 10^6$ | $1 \times 10^6$ | ml$^{-1}$ | [31] |
| $N_1$ | - | 1 | 1 | day$^{-1}$ | Estimated |
| $Q_1$ | - | $1 \times 10^2$ | $1 \times 10^2$ | ml$^{-1}$ | Estimated |
| $N$ | 50 | 50 | 50 | day$^{-1}$ | [14,32] |
| $N$ | 10 | 10 | 10 | day$^{-1}$ | [14] |

Figure 4. The dynamics of $B$, $M_r$, and $M_i$ with different values of $\beta_M$, in the three-equation model (13)-(15). The initial conditions are $B(0) = 100$, $M_r(0) = \alpha_M \mu_M$, and $M_i(0) = 0$. We set $\gamma_i = 0.01$ day$^{-1}$ and the others parameters from Table 3. As the rate of $M_r$ recruitment by $M_i$ and $B$ increasing from 0.1 to 10, the number of $M_i$ and $B$ decreases, while the number of $M_r$ is relatively stable.
Figure 5. Model 1: System (1)-(6) without involving Th1 cell and Treg cell. The initial conditions are $B(0) = 100$, $M_i(0) = \alpha M_i/\mu M_r$, and $M_a(0) = T_1(0) = T_r(0) = 0$. We set $\alpha_{T_1} = \alpha_{T_r} = \beta_{T_r} = 0 \text{ day}^{-1}$ and the other parameters from Table 3. Resting macrophages are infected by bacteria and become infected macrophages, leading to a sharp increase in bacteria.

The involvement of Treg cells may help control the number of Th1 cells, back to a homeostasis status; on the other hand, a strong Treg cell response may break the balanced situation to lose the control of bacterial growth.

For studying the effect of Th1 cells and Treg cells, and the interaction between macrophages and T cells, several regulation situations are considered with different settings in the model (1)-(6). Here we numerically solve the system by the built-in function ode23 solver in MATLAB with the ranges of the parameters estimated from the references [14, 31]. If the values of some parameters are not specified in the simulations, the values will be taken from Table 3, which corresponds to active disease status in the three-equation system.

Model 1: System without Th1 cell and Treg cell. First we consider the model (1)-(6) with initial conditions $B(0) = 100$, $M_r(0) = \alpha M_r/\mu M_r$, and $M_i(0) = M_a(0) = T_1(0) = T_r(0) = 0$. Figure 5 shows the simulations without Th1 and Treg cells involvement (by setting $\alpha_{T_1} = \alpha_{T_r} = \beta_{T_r} = 0$, we have $M_a(t) = T_1(t) = T_r(t) = 0$). Macrophages and Treg cells are not activated without Th1 cells so the system without Th1 cell involvement is the same as the three-equation system (13)-(15) we discussed in the previous section. The result shows that the population of bacteria $B$ loses control and the status becomes active disease.

Model 2: System with Th1 but without Treg cell. After Th1 cells are involved in the system, macrophages can be activated through Th1 cells and induce activated macrophages ($M_a$) which play a critical role in killing bacteria and enhance macrophage recruitment. Figure 6 shows the simulations with Th1 cell involvement but without Treg cell regulation (by setting $\alpha_{T_1} = 5.2 \times 10^{-2} \text{ day}^{-1}$, $\alpha_{T_r} = \beta_{T_r} = 0 \text{ day}^{-1}$, we have $T_r(t) = 0$). The population of bacteria reaches the maximum value after few months and then has a rapid drop. This oscillation continues and the maximum value of each oscillation finally reaches a bounded value. In this case, although the bacteria population is bounded, the individual status is not stable.
Figure 6. Model 2: System (1)-(6) with Th1 cell but without Treg cell regulation. The initial conditions are $B(0) = 100$, $M_r(0) = \alpha_{M_r}/\mu_{M_r}$ and $M_a(0) = T_1(0) = T_r(0) = 0$. We set $\alpha_{T_1} = 5.2 \times 10^{-2}$ day$^{-1}$, $\alpha_{T_r} = \beta_{T_r} = 0$ day$^{-1}$ and the other parameters from Table 3. Without regulation from Treg cells, the population of bacteria, macrophages, and Th1 cells oscillates continuously.

Model 3: System with Th1 cell and low level of Treg cell regulation. The oscillation in Figure 6 may be due to the strong Th1 response. Treg cell regulation may play a role in helping eliminate the oscillation through inhibiting the Th1 response. To measure how the regulation from Treg cell may control the oscillation in model 2, the Oscillation Index (OI) is introduced as follows

\[ OI = \frac{\max(B) - \min(B)}{\max(B) + \min(B)}, \]

where the maximum and the minimum are considered for the concentration of bacteria $B(t)$ between 3000 days and 5000 days. The larger the index, the stronger the oscillation presents.

First, we randomly generate 500 sets of parameters from their corresponding ranges shown in Table 3. Figure 7A shows the change of the average of the OI when Treg cells are involved by increasing $\alpha_{T_r}$ and $\beta_{T_r}$ from 0.01 to 1. When $\alpha_{T_r}$ and $\beta_{T_r}$ increase, the OI decreases and is finally close to zero. It supports that the increase of Treg cells could prevent Th1 from over-response, contributing to smaller oscillations than before. On the other hand, Figure 7B shows when $\alpha_{T_r}$ and $\beta_{T_r}$ is increasing from 0.01 to 0.1, the average of the maximum concentration of bacteria, $\max(B)$, are decreasing while Treg cells inhibit oscillations through reducing the
Figure 7. The effect of Treg cell regulation in Model 3. A) The change in the average of the OI with respect to the increases of $\alpha_{Tr}$, the $Tr$ activation rate by $Ma$ and $\beta_{Tr}$, the $Tr$ activation rate by $T1$; B) The change in the average of the maximum concentration of bacteria with respect to increases of $\alpha_{Tr}$ and $\beta_{Tr}$. Each dot represents an average of the simulations with 500 sets of randomly selected parameters from the ranges listed in Table 3. For each simulation, the initial conditions are $B(0) = 100$, $Mr(0) = \alpha_{Mr}/\mu_{Mr}$ and $Mi(0) = Ma(0) = T1(0) = Tr(0) = 0$. As $\alpha_{Tr}$ and $\beta_{Tr}$ increase, the oscillation of bacteria becomes smaller, while the maximum value of bacteria decreases first and then increases.

Th1 response; when $\alpha_{Tr}$ and $\beta_{Tr}$ are increasing from 0.1 to 1, the average of the maximum concentration of bacteria, max($B$), increases from $2 \times 10^3$ to $10^4$. It also shows that Treg cells regulation also has a disadvantage when the inhibition by Treg cell is too strong.

To be specific, the simulation shown in Figure 8 demonstrates that Treg cell regulation reduces the immune response to stabilize the dynamics of the system by setting $\alpha_{T1} = 5.2 \times 10^{-2}$ day$^{-1}$, $\alpha_{Tr} = \beta_{Tr} = 0.1$ day$^{-1}$ and $\beta_{Mr} = 0.01$ day$^{-1}$. Comparing with Figure 6, $Ma$ and $T1$ levels are reduced over 50% with Treg cell regulation, and $B$ and $Mi$ reaches a stable level after a few cycles of oscillation. This simulation shows that Treg cells can protect against overactivation of the immune response during TB infection.

Model 4: System with Th1 cell and high level of Treg cell regulation. As shown in Figure 7B, Treg cell regulation may lead to an active disease when the inhibition by Treg cell is too strong. To illustrate, we set $\alpha_{Tr} = \beta_{Tr} = 1$ day$^{-1}$ and other parameters as the values listed in Table 3. Figure 9 shows that the disease status becomes active with very high concentration of bacteria. Comparing with Model 3, as $\alpha_{Tr}$ and $\beta_{Tr}$ increase from 0.1 to 1, the disease status changes from latent form to active form, and the number of all macrophages, including $Ma$, $Mi$ and $Mr$ goes to zero after 1000 days. The status is relatively more unstable than that in Model 3.

As discussed in the previous section, strong macrophage recruitment (high value of $\beta_{Mr}$) may provide an inhibition of the activation of a latent disease even when
Figure 8. Model 3: System (1)-(6) with Th1 cell control and low level of Treg cell regulation. The initial conditions are $B(0) = 100$, $M_r(0) = \alpha_{M_r}/\mu_{M_r}$ and $M_i(0) = M_a(0) = T_1(0) = T_r(0) = 0$. We set $\alpha_{T_1} = 5.2 \times 10^{-2}$ day$^{-1}$, $\alpha_{T_r} = \beta_{T_r} = 0.1$ day$^{-1}$ and the other parameters from Table 3. With Treg cell regulation, the number of bacteria remains stable after a few cycles of oscillation.

Treg cell response is strong. To study the effect of strong macrophage recruitment when the regulation from Treg cell is strong, we fix $\alpha_{T_r} = \beta_{T_r} = 1$ day$^{-1}$ and increase the value of $\beta_{M_r}$ from 0.01 to 10 in log scale, with 500 sets of parameters randomly selected from their corresponding ranges listed in Table 3. Figures 10A,B show that macrophages inhibit the growth of bacteria, but they also cause oscillations when they reach a large amount. When $\beta_{M_r}$ increases from 0.01 to 0.4, the bacteria decline from a very high value to a small amount with relatively small OI and low bacterial concentration. It supports that the increase of macrophage recruitment has a significant contribution in controlling bacteria’s growth and makes the disease status latent even when Treg cell regulation is without control. As demonstrated in Figure 10C, by setting $\alpha_{T_r} = \beta_{T_r} = 1$ day$^{-1}$ and $\beta_{M_r} = 0.3$ day$^{-1}$, the effect of $M_r$ mitigates the strong inhibition of $T_r$, suppressing the growth of bacteria. Refering to (1)-(6), when $\beta_{M_r}$ is large, the growth rate of $M_r$ will increase. Then, the number of $M_a$ and $M_i$ will also increase, leading to an increase in Th1 cells. This interaction helps to mitigate the negative effects of the strong Treg cells’ inhibition and reduce the bacteria so that the system reaches a stable level.
However, when $\beta_M$ is larger than 0.4, increasing the value of $\beta_M$ causes stronger oscillations of the number of bacteria. Figures 10A shows that the average of the OI finally becomes 1 and Figures 10B shows that the average of the concentration of bacteria has an increase when $\beta_M > 0.4$. As shown in Figure 10D, we set $\alpha_T = \beta_T = 1$ day$^{-1}$ and $\beta_M = 3$ day$^{-1}$, the strong response from macrophages may cause high bacteria killing rate, then the numbers of macrophages and T cells go to zero rapidly and bacteria grows again, as the situation observed in Model 2.

6. **Discussion.** In this paper, we have built a mathematical model which consists of the dynamics of macrophages, Th1 and Treg cells, and the change of bacteria concentration during TB infection. Through separating the system into several simple subsystems, we obtained several stability conditions corresponding to different disease situations.

Our results supported by numerical simulations showed that Th1 cells and Treg cells play critical roles in TB infection: Th1 cells inhibit the number of infected macrophages to reduce the portion of bacteria released from infected macrophages; Treg cell regulation reduces the immune response to stabilize the dynamics of the system.
In this paper, we considered several situations with different regulation controls. While no Th1 and Treg cells are involved (Model 1), the number of bacteria blows up to infinity. It indicates that Th1 and Treg cells may play an important role in controlling the growth of bacteria by activating macrophages to kill them. Although Th1 cells could control the growth of bacteria in a short time by inhibiting
the number of infected macrophages and activating resting macrophages, the imbalance immune system (without regulation from Treg cells) may cause the oscillatory behavior of disease population.

While Th1 cells are introduced (Model 2), the number of bacteria would decrease drastically in a short time but increase again later. The fluctuation of bacteria, as well as other macrophages' and Th1 cells' populations, continues for a long time period. This oscillation could be explained by the over-activation of macrophages by Th1 cells. Without inhibition from regulatory T cells, Th1 cells grow fast, leading to rapid activation from resting macrophages to active macrophages which enhance killing bacteria. In turn, a large number of active macrophages increases the proliferation of macrophages; therefore, more macrophages are infected by bacteria and the population of bacteria increases again. This dynamic process repeats many times and the oscillatory behavior may continue over time.

Recent clinical and experimental results support that latency is maintained by a balance between pathogen persistence and the immune response [18]. Regulatory T cells play an essential role in immune homeostasis. When Treg cells are involved in down-regulating the immune response against Mtb infection (Model 3), the oscillatory behavior can be avoided by suppressing Th1 cells and inhibiting the activation of resting macrophages. Thus Treg cells allow the persistence and the establishment of a chronic infection, keeping a fine balance in our immune system [18, 23]. Our results support that Th1 cells' response with the regulation of Treg cells can maintain immune homeostasis and thus maintain latency during TB infection.

Nevertheless, strong Treg cells' response may also be involved in the reactivation of bacteria, thus leading to an activation of the latent disease. By increasing the Treg regulation level, the disease status jumps from latent to active. Recent data in vitro and in vivo using two-photon microscopy suggest that Treg cells limit Th1 cells' signal duration [25]. Through controlling Th1 cells, strong Treg cells' response can inhibit macrophage activation and increase infected macrophages; thus more bacteria are released from infected macrophages and grow faster. Also, our results suggest that the macrophage recruitment may have a critical role in this interaction. When the rate of macrophage recruitment increases, even Treg cells are at a high level, the disease status remains latent.

For simplicity, the effects of cytokines were implicitly modeled in terms of some parameters and the dynamics of cytokines were not discussed in our study. In medical applications, the dynamics of cytokines can be used for determining the disease situation of a patient. Our model can be extended to include the dynamics of several important cytokines, for example, IL-10, IL-12, IL-4, TGF-β and IFN-γ, and the stability analysis in our study can provide a hint for studying the extended model of TB infection.

In summary, this work examines the TB infection thoroughly and draws the following conclusions:

- Infected macrophages and activated macrophages will induce the activation and production of Th1 cells, thus the amount of Th1 increases. It consistent with the experimental results in [30], finding that the frequency of T cell activation increases in active TB.
- According to [18], active TB patients have more Treg cells than uninfected individuals. Indeed, Treg cells increase and help maintain balance by controlling Th1 response during TB infection.
• Enhanced macrophage feedback could help control the over-reaction of Treg cells, thus maintain latency.

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Appendix A. Proofs of the theorems.

A.1. Proof of Theorem 1. First we consider the case that \( M_r(0) \geq \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \) and \( B(0) > 0 \). From the equation, it is easy to see that if \( B(0) > 0 \) and \( M_r(0) \geq 0 \), the value of \( B(t) \) never goes to zero with a finite time \( t \) and the value of \( M_r(t) \) never becomes negative. With the condition provided in Theorem 1, we obtain \( \frac{dM_r}{dt} = f_M(B(t), M_r(t)) < \alpha_{M_r} - \mu_{M_r}, \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} < 0 \) for any \( B(t) > 0 \) and \( M_r(t) \geq \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \). It implies that there exists a positive value \( T \) such that when \( t > T \), \( M_r(t) < \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \) and \( B(t) > 0 \). Without loss of generality, we assume that \( M_r(0) < \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \) and \( B(0) > 0 \).

The analysis above implies that \( M_r(t) < \frac{\alpha_B}{k_{BM} + k_{MB}(N/2)/K_B} \) and \( B(t) > 0 \) for any \( t > 0 \). Hence the derivative

\[
\frac{dB}{dt} = f_B(B(t), M_r(t)) > B(t) \left( \alpha_B - \frac{\alpha_B (k_{BM} + k_{MB}(N/2)/B(t) + K_B)}{k_{BM} + k_{MB}(N/2)/K_B} \right) > 0
\]

for any \( t > 0 \). We show that \( B(t) \) is increasing and

\[
\frac{dB}{dt} > B(0) \left( \alpha_B - \frac{\alpha_B (k_{BM} + k_{MB}(N/2)/B(0) + K_B)}{k_{BM} + k_{MB}(N/2)/K_B} \right) > 0
\]

for all \( t > 0 \). Overall, \( B(t) \) will go to infinity when \( t \) tends to infinity.

A.2. Proof of Theorem 2. First we consider the case that \( M_r(0) \leq \frac{\alpha_B}{k_{BM}} \). With the condition provided in Theorem 2, we obtain

\[
\frac{dM_r}{dt} = f_M(B(t), M_r(t)) > \left( \alpha_{M_r} - (k_{MB} + \mu_{M_r}) \frac{\alpha_B}{k_{BM}} \right) > 0
\]

for any \( M_r(t) \leq \frac{\alpha_B}{k_{BM}} \). So there exists a positive value \( T \) such that \( M_r(t) > \frac{\alpha_B}{k_{BM}} \) when \( t > T \). Without loss of generality, we assume that \( M_r(0) > \frac{\alpha_B}{k_{BM}} \) and \( B(0) \geq 0 \).

The analysis above implies that \( M_r(t) > \frac{\alpha_B}{k_{BM}} \) and \( B(t) \geq 0 \) for any \( t > 0 \). In the case, \( \frac{dB}{dt} = f_B(B(t), M_r(t)) < B(t) (\alpha_B - k_{BM} M_r(0)) < 0 \). By Grönwall’s inequality, we obtain that \( B(t) \) will tend to zero when \( t \) becomes large.

For showing \( M_r(t) \to \alpha_{M_r}/\mu_{M_r} \) as \( t \to \infty \), we consider a function

\[
V(B(t), M_r(t)) = (M_r - \alpha_{M_r}/\mu_{M_r})^2 + \Omega B \text{ for } B(t) \geq 0 \text{ and } M_r(t) > \frac{\alpha_B}{k_{BM}}
\]

where \( \Omega = 2 \left( \frac{\alpha_B}{\mu_{M_r}} - \frac{\alpha_M}{\mu_{M_r} + k_{MB}} \right) \frac{k_{BM} + \alpha_B}{\alpha_B(N/2) \alpha_{M_r}} + 1 \geq 0 \). It is easy to show that \( V \geq 0 \) for \( B(t) \geq 0 \) and \( M_r(t) > \frac{\alpha_B}{k_{BM}} \), and \( V = 0 \) if and only if \( B = 0 \) and \( M_r = \alpha_{M_r}/\mu_{M_r} \).

When we consider a derivative of \( V \) respect to \( t \), we obtain

\[
\frac{dV}{dt} = 2 \left( M_r(t) - \frac{\alpha_{M_r}}{\mu_{M_r}} \right) \left( \alpha_{M_r} - k_{MB} M_r(t) \frac{B(t)}{B(t) + K_B} - \mu_{M_r} M_r(t) \right) + \Omega \left( \alpha_B B(t) - k_{BM} M_r(t) B(t) - k_{MB} \frac{N}{2} M_r(t) \frac{B(t)}{B(t) + K_B} \right).
\]

(23)
For the case that \( B(t) = 0 \), the derivative is negative if and only if \( M(t) \neq \alpha_{M_r}/\mu_{M_r} \). For the case that \( B(t) > 0 \), the second term of (23) is always negative in the region of \( M_r(t) > \frac{\alpha_B}{k_{BM}} \).

For analyzing the second term, we divide into three cases:

- If \( M_r(t) \geq \frac{\alpha_{M_r}}{\mu_{M_r}} \), the first term (23) is non-positive so \( \frac{dV}{dt} \) is strictly negative;

- If \( \frac{\alpha_B}{k_{BM}} < M_r(t) < \frac{\alpha_{M_r}}{\mu_{M_r} + k_{MB}/\mu_{M_r} + k_B} \), the first term (23) is negative so \( \frac{dV}{dt} \) is strictly negative;

- If \( \frac{\alpha_M}{\mu_{M_r} + k_{MB}/\mu_{M_r} + k_B} \leq M_r(t) < \frac{\alpha_{M_r}}{\mu_{M_r}} \), we can obtain the following inequality
  \[
  \frac{dV}{dt} \leq 2\left( \frac{\alpha_{M_r}}{\mu_{M_r}} - \frac{\alpha_{M_r}}{\mu_{M_r} + k_{MB}} \right) \left( k_{MB} \frac{\alpha_{M_r}}{\mu_{M_r}} B(t) + k_B \right) - \Omega \left( k_{MB} \frac{N \alpha_B}{2 k_{BM}} B(t) + k_B \right)
  \]

Overall, \( \frac{dV}{dt} < 0 \) in the set \( \{(B, M_r)|B \geq 0 \text{ and } M_r > \frac{\alpha_B}{k_{BM}}\} \) except \( (0, \alpha_{M_r}/\mu_{M_r}) \). We can prove that \( V \to 0 \) as \( t \to \infty \) and it implies that the solution of \( (B(t), M_r(t)) \) will go to the steady state \( (0, \alpha_{M_r}/\mu_{M_r}) \) when \( t \) tends to infinity.

**A.3. Proof of Theorem 3.**

**Proof of part 1.** For the part 1 of the theorem, we are going to show that if \( 0 \leq B(0) < B^* \) and \( f_B(B(0), M_r(0)) < 0 \) (Area 1 in Figure 3C), \( B(t) \) and \( M_r(t) \) will remain in the area of \( 0 \leq B(t) < B^* \) and \( f_B(B(t), M_r(t)) < 0 \) (Area 1) for all \( t > 0 \).

Since \( \frac{dB}{dt} = f_B(B, M_r) < 0 \) in the Area 1, \( B(t) \) keeps decreasing in that area so \( (B(t), M_r(t)) \) cannot leave the Area 1 by crossing \( B = B^* \).

If \( f_B(B(t), M_r(t)) = 0 \) and \( B(t) < B^* \), we have

\[
\frac{df_B(B(t), M_r(t))}{dt} = \frac{\partial f_B}{\partial B} f_B(B(t), M_r(t)) + \frac{\partial f_B}{\partial M} f_M(B(t), M_r(t))
\]

so \( (B(t), M_r(t)) \) cannot leave the Area 1 by crossing \( f_B(B(t), M_r(t)) = 0 \).

With the fact that \( B(t) \) could not be negative with non-negative initial values, we show that if \( 0 \leq B(0) < B^* \) and \( f_B(B(0), M_r(0)) < 0 \), \( B(t) \) and \( M_r(t) \) will remain in the area of \( 0 \leq B(t) < B^* \) and \( f_B(B(t), M_r(t)) < 0 \) (Area 1).

By considering the same method used in the proof of Theorem 2 and the function

\[
V(B(t), M_r(t)) = (M_r - \alpha_{M_r}/\mu_{M_r})^2 + \Omega B \text{ for } 0 \leq B(t) < B^* \text{ and } f_B(B(t), M_r(t)) < 0,
\]

where \( \Omega = 2\left( \frac{\alpha_{M_r}}{\mu_{M_r}} - \frac{\alpha_{M_r}}{\mu_{M_r} + k_{MB}} \right) \left( \frac{k_{BM}}{\mu_{M_r}} \frac{\alpha_{M_r}}{\mu_{M_r}} \right) + 1 \geq 0 \), we can prove that \( (B(t), M_r(t)) \to (0, \alpha_{M_r}/\mu_{M_r}) \) as \( t \to \infty \).

**Proof of part 2.** First we will show that if \( B(0) > B^* \) and \( f_B(B(0), M_r(0)) > 0 \) (Area 2 in Figure 3C), \( B(t) \) and \( M_r(t) \) will remain in the area of \( B(t) > B^* \) and \( f_B(B(t), M_r(t)) > 0 \) (Area 2) for all \( t > 0 \).

Since \( \frac{dB}{dt} = f_B(B, M_r) > 0 \) in the Area 2, \( B(t) \) keeps increasing in that area so \( (B(t), M_r(t)) \) cannot leave the Area 2 through \( B = B^* \).
If $f_B(B(t), M_r(t)) = 0$ and $B(t) > B^*$, we have
\[
\frac{df_B(B(t), M_r(t))}{dt} = \frac{\partial f_B}{\partial B} f_B(B(t), M_r(t)) + \frac{\partial f_B}{\partial M} f_M(B(t), M_r(t))
\]
so $(B(t), M_r(t))$ cannot leave the Area 2 by crossing $f_B(B(t), M_r(t)) = 0$.

If $M_r(t) = 0$ and $B(t) > B^*$, $\frac{dM_r}{dt} = f_M(B(t), M_r(t)) = \alpha_{M_r} > 0$ so $(B(t), M_r(t))$ cannot leave the Area 2 by crossing $M_r(t) = 0$.

Overall, we show that if $B(0) > B^*$ and $f_B(B(0), M_r(0)) > 0$, $B(t)$ and $M_r(t)$ will remain in the area of $B(t) > B^*$ and $f_B(B(t), M_r(t)) > 0$ (Area 2). By applying the method used in the proof of Theorem 1, we can prove that $B(t) \to \infty$ as $t \to \infty$. □

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