H.M. Yang (2012). Mathematical Modelling of the Interaction between Mycobacterium tuberculosis Infection and Cellular Immune Response. In
(Editor R. Mondaini): Proceedings of BIOMAT 2011 (Santiago, Chile).
World Scientific: Singapore: 309-330 (ISBN 13 978-981-4343-42-8)

MATHEMATICAL MODELLING OF THE INTERACTION BETWEEN *Mycobacterium tuberculosis* INFECTION AND CELLULAR IMMUNE RESPONSE*

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In general, cellular immune response results in the suppression of mycobacterial infection, but does not completely eradicate it. This is the reason why the majority of cases (95%) limits proliferation of the bacilli and produces a long-lasting partial immunity, and 5% of infected individuals develop early progressive disease that occurs within 2-5 years of infection. One of the characteristics of *Mycobacterium* infection is the replication of the bacteria inside of alveolar macrophages. For this reason 5% of asymptomatic individuals have late disease, which is caused by endogenous reactivation as long as several decades after infection. We develop a simple mathematical model that describes the interaction between *Mycobacterium tuberculosis* and cellular immune response. The risk of tuberculosis reactivation is determined by granulomas formation on one side and cellular immune response to phagocytize bacteria and to destroy granuloma on other side. The model is analyzed in the initial phase of mycobacterial infection, and an associated risk of tuberculosis among tobacco smokers is established.

1. Introduction

Mycobacterium tuberculosis (Mtb) infects one third of the world's population and causes 8 million of new cases of tuberculosis (TB) and approximately 2 million deaths each year.[1] In 2006 new cases of TB were estimated in 9.2 million, causing nearly 1.6 million deaths.[2] TB is the most important cause of adult death due to infectious disease after HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome). The two factors essential for its rapid spread are crowded living conditions and a population with little native resistance. For this reason, TB has become

*This work is supported by fapesp – projeto temático
†Work partially supported by grant from cnpq. Corresponding author.

concentrated in certain medically underserved populations: the urban poor, alcoholic, intravenous drug users, the homeless, migrant farmer workers and prison inmates.[3]

TB is the prototype of infections that require a cellular immune response for their control, although abundant antibodies are also produced during the infection, but they play no apparent role in the defense mechanism.[4] In the first few weeks after the exposure, the host has almost no immune defense against Mtb infection, and unrestrained replication proceeds for weeks, both in the initial focus (alveolar spaces or within alveolar macrophages) and in lymphohematogeneous metastatic foci. Within 3 to 9 weeks after infection, tissue hypersensitivity becomes manifest, when enhanced macrophage mycobactericidal activity (or cellular immunity) appears. For instance, when both antigen load and degree of tissue hypersensitivity are high, lymphocytes and macrophages are present in a less organized fashion, and tissue necrosis may be present, which tends to be incomplete, resulting in a solid or semisolid acellular and amorphous material referred to as caseous because of its cheesy consistency. The chemical environment and oxygen tension in solid caseous material tend to inhibit microbial multiplication; however, it is inherently unstable, especially in the lungs, where it tends to liquefy and discharge through the bronchial tree, producing a tuberculous cavity and providing conditions in which bacterial population increases.[3]

TB is a directly transmitted infection that presents two routes for the evolution to disease due to the immune response: direct progression (the disease develops soon after infection) or endogenous reactivation (the disease can develop many years after infection). After primary infection, progressive TB may develop either as a continuation of primary infection or as an endogenous reactivation of a latent focus. Factors regarding to the reactivation are malnutrition and weakening of the immune system. In some patients, however, disease may also result from exogenous reinfection by a second strain of Mtb.[5] In this paper we propose a simple model taking into account the interplay between Mtb infection and cellular immune response in order to explore the mechanisms behind early progression (fast) and late reactivation (slow) of TB. Here, we analyze the initial phase of the interaction between Mtb and immune response, which is relevant to understanding different infection outcomes. In a companion paper, we analyze the effects of granuloma formation from alveolar macrophages and further release of mycobacteria in bronchial tree by tuberculoids.[6]

The paper is structured as follows. In section 2 a simple mathematical

model of primary TB and endogenous reactivation of TB is developed. In section 3 the direct progression to TB model is analyzed, and numerical results are given in section 4. Conclusion is presented in section 5.

2. Model formulation

Following Mtb infection, the bacillus is phagocytosed by an alveolar macrophage. Once the mycobacteria are internalized by inactivated macrophages they continue to proliferate by evading intracellular killing mechanisms. T-cells are activated by recognition and presentation of the antigenic epitopes of the microorganism, and they release cytokines, which lead to a state of macrophage activation and granuloma formation that, in the majority of cases, result in the suppression of mycobacterial proliferation. The principal defense of the human host against a Mtb infection is the formation of granulomas, organized collections of activated macrophages, including epithelioid and multinucleated giant cells, surrounded by lymphocytes. This granuloma can sequester and contain the bacteria preventing active disease, and if the granuloma is maintained, these bacteria may remain latent for a person's lifetime. Secretion of a variety of chemoattractant cytokines following phagocytosis of the bacilli by the macrophage is critical not only to the formation of the granuloma but also to its maintenance.[7] In over 95% of cases (5% of patients progress to disease within 2-5 years of infection) this immune response achieves the containment of Mtb but does not completely eradicate it. This leaves the person infected with bacilli, and during the period of containment (latent infection) individuals are asymptomatic and noninfectious. Among these individuals, 5% progress to disease during the remainder of their life. These numbers are dramatically different in patients who have compromised cell-mediated immune systems.[8]

Our objective is the development of a simple mathematical model to describe the evolution of TB,[9−10] taking into account the initial immune response mounted against Mtb infection, which is relevant to understanding different infection outcomes. The progression from latent infection to active disease is dictated by the balance between the virulent properties of the organisms and the host defense. In this model, we did not include every cell potentially involved (NK cells, eosinophils, B cells, giant cells, etc.) in the immune response. The simple model presented here does not consider the production of cytokines (the actions of cytokines that down- and up-regulate the immune response are summarized by the quantity of Mtb in the local of infection), assuming that the recruitment (migration)

and proliferation of immune response cells are simply proportional to Mtb quantity. Also, other cells, as NK cells, eosinophils, B cells and giant cells are assumed as being part of the macrophage population.

The invading Mtb (designed as B, the concentration of free bacilli at time t) is phagocytized by an alveolar macrophage (designed as M, the concentration of inactivated macrophages at time t), resulting in granuloma formation (designed as G, the concentration of granulomas at time t). The interaction of T-cell (designed as L, the concentration of inactivated lymphocytes at time t) with mycobacterium (antigenic epitopes) results in activated T-cells (designed as L_a, the concentration of activated lymphocytes at time t), which lead to a state of macrophage activation (designed as M_a, the concentration of activated macrophages at time t). The inactivated macrophages that engulfed bacteria (reactive granulomas, G) can be activated by activated lymphocytes, or destroyed by activated macrophages in the early phase. When they are destroyed by activated macrophages, they become unreactive granulomas (designed as G_n, the concentration of unreactive granulomas at time t). The reactive granulomas become solid caseous materials (designed as T, the concentration of tuberculoids at time t), and they stay in the solid phase during a variable period of time, which characterizes the latent infection. The actions of other components of immune system (as cytokines, which productions are assumed to be proportional to bacterial load) are captured by the model's parameters.[7]

The infecting mycobacteria are under unrestrained replications because the host has almost no immune defense against them, which is described by the parameter α (dimension $time^{-1}$), the intrinsic growth rate. However, the constraint with the nutrients and alveolar spaces restrict their multiplication, with the carrying capacity given by k (dimension $[B]$, where $[\bullet]$ is the concentration, that is, number/volume). The per-capita growth rate of bacteria is then $\alpha\,(1 - B/k)$, and μ_B (dimension $time^{-1}$) is designed to the per-capita natural mortality (unviable or those captured and destroyed by surrounding tissues) rate of bacteria. The rates at which free bacteria are phagocytized by inactivated (to form granulomas) and activated (to be destroyed by lysis) macrophages are, respectively, β_B and δ_B (dimensions $[M]^{-1}/time$). The inactivated macrophages and lymphocytes are produced at constant rates λ_M (dimension $[M]/time$) and λ_L (dimension $[L]/time$), and suffer per-capita mortality rates of μ_M and μ_L (dimensions $time^{-1}$), respectively. The rates of activation of macrophages and lymphocytes are ε_M (dimension $[L]^{-1}/time$) and ε_L (dimension $[M]^{-1}/time$, which encompasses the action of antigen presenting dendritic cells considered here being

proportional to the activated lymphocytes), and the rates of recruitment of activated macrophages (from activated monocytes) and proliferation of lymphocytes are γ_M and γ_L (dimensions $[B]^{-1}/time$), respectively. The rates of recruitment of inactivated macrophages (from inactivated monocytes) and naive lymphocytes to the local of infection are r_M (dimension $[B]^{-1}/time$) and r_L (dimension $[L][M]^{-1}[B]^{-1}/time$), respectively.

Inactivated macrophages engulf approximately n^{-1} (dimension of n is $[M]/[B]$) bacilli and become granulomas. In the early phase, granulomas can be activated by activated lymphocytes and also destroyed by activated macrophages. The activation occurs at a per-capita rate ε_G (dimension $[L]^{-1}/time$), and the dwelling bacteria in the reactive granulomas are destroyed at a rate δ^* (dimension $[M]^{-1}/time$, that is, $[G]=[M]$), becoming unreactive granuloma. The reactive and unreactive granulomas are under μ_G (dimension $time^{-1}$) per-capita mortality rate. The reactive granulomas become tuberculoids (solid caseous material) at a rate θ (dimension $time^{-1}$), which are liquefied at a rate ϕ (dimension $time^{-1}$), and discharge on average f (dimension $[B]/[M]$, that is, $[T]=[M]$) bacteria in the bronchial tree. The tuberculoids are under μ_T (dimension $time^{-1}$) per-capita mortality rate, and they are destroyed and release bacteria when the environment becomes suitable.

The dynamics of the interaction between Mtb and immune system is described by the following system of equations

$$\begin{cases} \frac{d}{dt}B &= \alpha\left(1-\frac{B}{k}\right)B-\mu_B B-\beta_B MB-\delta_B M_a B+fh\left(t-t_1\right)h\left(t_2-t\right)\phi T \\ \frac{d}{dt}M &= \lambda_M-\mu_M M-\varepsilon_M ML_a-n\beta_B MB+r_M L_a B \\ \frac{d}{dt}M_a &= \varepsilon_M ML_a-\mu_M M_a+\gamma_M M_a B+\varepsilon_G GL_a \\ \frac{d}{dt}L &= \lambda_L-\mu_L L-\varepsilon_L LM_a+r_L L_a B \\ \frac{d}{dt}L_a &= \varepsilon_L LM_a-\mu_L L_a+\gamma_L L_a B \\ \frac{d}{dt}G &= n\beta_B MB-\varepsilon_G GL_a-\mu_G G-\theta G-\delta^* GM_a \\ \frac{d}{dt}T &= \theta G-\mu_T T-h\left(t-t_1\right)h\left(t_2-t\right)\phi T \\ \frac{d}{dt}G_n &= \delta^* GM_a-\mu_G G_n, \end{cases} \tag{1}$$

where the Heaviside or step function $h(z)$ is such that $h(z)=0$, if $z<0$, and $h(z)=1$, if $z\geq 0$. We are assuming that at $t=t_1$ occurred changes in the immune system of the host (as immunosuppression) and/or physiological conditions become worse, which conditions persist up to t_2, with $0\leq t_1<t_2$.

In order to deal with an insightful system and obtain some analytical results, we perform the following simplifications. Firstly, we disregard the activation of early stage of granulomas ($\varepsilon_G=0$), and the equation for

granulomas G can be written as $\frac{d}{dt}G = n\beta_B MB - \mu_G G - \theta G - \delta^* G M_a$. Now, let us join the macrophage sub-populations in one class, that is, $C = M + M_a$. By summing up the second and third equations of (1), we obtain $\frac{d}{dt}C = \lambda_M - \mu_M C + (\gamma_M + r_M) M_a B - n\beta_B MB$. As a consequence of introducing new variable C, the fourth and fifth equations become decoupled from the system, and the equation for C does not depend on the lymphocytes population that activates macrophages and suppresses the factor (ε_M) of transferring macrophages from naive to activated status. In order to introduce the action of cell mediated immune response, let us suppose that the quotient between M and M_a provides indirectly the action of activated lymphocytes, that is, the quantity q, defined by

$$q = \frac{M_a}{M + M_a} = \frac{M_a}{C},$$

measures how the cell mediated response is strongly acting to eliminate the infection. The parameter q takes into account the activation (ε_L) and recruitment (r_L and γ_L) of lymphocytes, and also the activation of macrophages (ε_M). Hence, we have $M_a = qC$ and $M = (1-q)\,C$, with $0 \leq q \leq 1$. Finally, the last equation can be dropped out from the system.

Therefore, substituting $\beta = (1-q)\,\beta_B$, $\varepsilon = q\delta_B$, $\lambda = \lambda_M$, $\mu_C = \mu_M$, $\gamma = q\,(\gamma_M + r_M)$ and $\delta = q\delta^*$, we achieve the simplified system of equations

$$\begin{cases} \frac{d}{dt}B = \alpha\left(1 - \frac{B}{k}\right)B - \mu_B B - (\beta + \varepsilon)\,CB + fh\,(t - t_1)\,h\,(t_2 - t)\,\phi T \\ \frac{d}{dt}C = \lambda - \mu_C C + (\gamma - n\beta)\,CB \\ \frac{d}{dt}G = n\beta CB - (\mu_G + \theta)\,G - \delta CG \\ \frac{d}{dt}T = \theta G - \mu_T T - h\,(t - t_1)\,h\,(t_2 - t)\,\phi T. \end{cases} \tag{2}$$

The parameters λ and μ_C are new representations of, respectively, λ_M and μ_M defined above. We recall that the class C is compounded by all cells of the immune system and is generically referred to as macrophages.

The parameters ε, γ, δ and β encompass the action of cytokines and dendritic cells that mediate the immune response (q increases, increasing M_a). The strength of the immune response can be assessed by the capacity of the lymphocytes activation, q. Increasing the immune response (q), the formation of granulomas (β) is decreased linearly from β_B (the maximum engulfment resulting when all macrophages are inactivated) up to zero, while the destruction of them (δ), engulfment and lysis of bacteria (ε) and the recruitment of activated macrophages (γ) increase linearly from zero up to, respectively, δ^*, δ_B and $(\gamma_M + r_M)$, which are the limiting capacity of lymphocytes induction (there are not inactivated macrophages).

The system of equations (2) is analyzed as autonomous (direct progression to TB) and non-autonomous (endogenous reactivation of TB, which is left to a future work[6]) dynamical systems. We determine the equilibrium points $Q = (\bar{B}, \bar{C}, \bar{G}, \bar{T})$ and perform the stability analysis.

3. Direct progression to TB Model

In 5% of cases, the patients progress to disease within 2-5 years of infection. Let us analyze the absence of endogenous reactivation, that is, we let $t_1 \to \infty$ in the system of equations (2), and obtain

$$\begin{cases} \frac{d}{dt}B = \alpha\left(1 - \frac{B}{k}\right)B - \mu_B B - (\beta + \varepsilon)\,CB \\ \frac{d}{dt}C = \lambda - \mu_C C + (\gamma - n\beta)\,CB, \end{cases} \tag{3}$$

and two decoupled equations for granulomas and tuberculoids $\frac{d}{dt}G = n\beta CB - (\mu_G + \theta)\,G - \delta CG$ and $\frac{d}{dt}T = \theta G - \mu_T T$. These equations have as equilibrium values

$$\bar{G} = \frac{n\beta\bar{C}\bar{B}}{\mu_G + \theta + \delta\bar{C}} \quad and \quad \bar{T} = \frac{\theta}{\mu_T}\frac{n\beta\bar{C}\bar{B}}{\mu_G + \theta + \delta\bar{C}}, \tag{4}$$

where $\bar{B}$ and $\bar{C}$ are equilibrium values of Mtb and macrophages.

In the direct progression to TB, the analysis of the model is very simple: It is enough to analyze the system (3) with only two equations for B and C. The equilibrium point $Q = \left(\bar{B}, \bar{C}, \bar{G}, \bar{T}\right)$ is composed by $\bar{B}$ and $\bar{C}$ obtained from equation (3), and $\bar{G}$ and $\bar{T}$ using equation (4). These latter values always exist whenever $\bar{B}$ and $\bar{C}$ are feasible, which is the reason for not considering $\bar{G}$ and $\bar{T}$ in all analyses. Hence, we determine the equilibrium point $P = \left(\bar{B}, \bar{C}\right)$, which is a subset of the equilibrium Q, and assess its stability. The local stability of the equilibrium point $P = \left(\bar{B}, \bar{C}\right)$ is assessed by the eigenvalues of Jacobian matrix

$$J = \begin{bmatrix} (\alpha - \mu_B) - \frac{2\alpha}{k}\bar{B} - (\beta + \varepsilon)\,\bar{C} & -(\beta + \varepsilon)\,\bar{B} \\ (\gamma - n\beta)\,\bar{C} & -\mu_C + (\gamma - n\beta)\,\bar{B} \end{bmatrix}.$$

The eigenvalues are roots of the second order polynomial

$$\Lambda(\phi) = \phi^2 + b_1\phi + b_0, \tag{5}$$

where the coefficients are

$$\begin{cases} b_1 = \mu_C - (\alpha - \mu_B) - (\gamma - n\beta)\,\bar{B} + \frac{2\alpha}{k}\bar{B} + (\beta + \varepsilon)\,\bar{C} \\ b_0 = \left[(\alpha - \mu_B) - \frac{2\alpha}{k}\bar{B} - (\beta + \varepsilon)\,\bar{C}\right]\left[-\mu_C + (\gamma - n\beta)\,\bar{B}\right] + (\gamma - n\beta)\,(\beta + \varepsilon)\,\bar{C}\bar{B}. \end{cases} \tag{6}$$

According to the Routh-Hurwitz criteria for second degree polynomial, $\Lambda(\phi)$ has negative real part if $b_1 > 0$ and $b_0 > 0$.[11] The system of equations (3)

does not present closed orbits. Calling $\frac{d}{dt}B = F(B,C)$ and $\frac{d}{dt}C = G(B,C)$, and using a function $A(B,C) = 1/(BC)$, we have

$$\frac{\partial}{\partial B}\left[A(B,C)F(B,C)\right] + \frac{\partial}{\partial C}\left[A(B,C)G(B,C)\right] = -\frac{\alpha}{kC} - \frac{\lambda}{BC^2},$$

which is not identically zero and does not change sign in $\Omega = \left\{(B,C) \in R^2 \mid B > 0; C > 0\right\}$. Hence, according to Dulac's criterion,[12] there are not closed orbits in this region.

First, the trivial equilibrium point of the system of equations (3) is $P_0 = (0, C_0)$, where

$$C_0 = \frac{\lambda}{\mu_C} \tag{7}$$

is the macrophage population at equilibrium, because they are produced constantly by bone marrow to replenish those removed. To assess the local stability of the trivial equilibrium point $P_0 = (0, C_0)$, we calculate the eigenvalues of the characteristic polynomial (5) with the coefficients (6) given by $b_1 = \mu_C - (\alpha - \alpha_0)$ and $b_0 = -\mu_C(\alpha - \alpha_0)$. They are $\phi_1 = -\mu_C$ and $\phi_2 = \alpha - \alpha_0$, with α_0 being given by

$$\alpha_0 = \mu_B + (\beta + \varepsilon)\frac{\lambda}{\mu_C}, \tag{8}$$

which is the threshold of the intrinsic growth rate of mycobacterium α. Hence P_0 is locally asymptotically stable (LAS) when $\alpha < \alpha_0$ (stable node), when $\alpha = \alpha_0$, stable along $C = C_0$, and otherwise, saddle point.

The trivial equilibrium P_0 is LAS when $\alpha < \alpha_0$, regardless the relative values between γ and $n\beta$. Let us analyze the global stability. Let us define a Lyapunov function in $V : R_+^2 \to R$ given by $V = B$, whose orbital derivative is

$$\dot{V} = \left\{\alpha - \mu_B - (\beta + \varepsilon)C - \frac{\alpha}{k}B\right\}B, \tag{9}$$

or, adding and subtracting C_0,

$$\dot{V} = \left\{\alpha - \left[\mu_B + (\beta + \varepsilon)\frac{\lambda}{\mu_C}\right] - (\beta + \varepsilon)(C - C_0) - \frac{\alpha}{k}B\right\}B. \tag{10}$$

The global stability is analyzed according to the relative values between γ and $n\beta$ (see below).

The average number of secondary bacteria produced by one invading Mtb is defined as

$$R_0 \equiv \frac{\alpha}{\alpha_0} = \frac{\alpha}{\mu_B + (\beta + \varepsilon)\frac{\lambda}{\mu_C}}, \tag{11}$$

showing that if $R_0 > 1$, mycobacteria can be settled at an infective level, because the trivial equilibrium P_0 is unstable for $\alpha > \alpha_0$. Note that $(\alpha_0)^{-1}$ is the average period of surviving time of free bacteria, which is decreased by the remotion of bacteria by granuloma formation and phagocytosis promoted by activated macrophages, both depending on the concentration of macrophages. Hence, R_0 is the average number of bacteria replicated from one invading bacterium. Being the free mycobacteria decreased by granuloma formation (β) and when phagocytized by activated macrophages (ε), mycobacterium must increase the ability to fit and to proliferate rapidly (α must surpass the critical value α_0) in order to overcome those opposing factors, which increases with increasing amount of macrophages (λ/μ_C).

When $\beta = \varepsilon = 0$, we have the situation in which mycobacteria replicate unrestrained because the immune system is not acting. The non-interacting system (3) has the equilibrium point $P_{non} = (B_0, C_0)$, where B_0, given by

$$B_0 = \frac{k}{\alpha}(\alpha - \mu_B), \tag{12}$$

is the maximum free bacilli ($\bar{B} \leq B_0$). Notice that P_{non} is stable when $\alpha < \mu_B$.

Now, the non-trivial equilibrium point of the system of equations (3), $P^* = \left(\bar{B}, \bar{C}\right)$, has the coordinates given by

$$\bar{C} = \frac{\lambda}{\mu_C - (\gamma - n\beta)\bar{B}} \tag{13}$$

and $\bar{B}$, which is the positive solution of the second order polynomial $f\left(\bar{B}\right)$ given by

$$f\left(\bar{B}\right) = a_2\bar{B}^2 + a_1\bar{B} + a_0, \tag{14}$$

with coefficients $a_2 = \frac{\alpha}{k}(\gamma - n\beta)$, $a_1 = -\left[\frac{\alpha}{k}\mu_C + (\alpha - \mu_B)(\gamma - n\beta)\right]$ and $a_0 = \mu_C(\alpha - \alpha_0)$; and discriminant $\Delta_f = \left[\frac{\alpha}{k}\mu_C - (\alpha - \mu_B)(\gamma - n\beta)\right]^2 + 4\frac{\alpha}{k}(\gamma - n\beta)(\beta + \varepsilon)\lambda$. Depending on the values assigned to the model's parameters, the polynomial $f\left(\bar{B}\right)$ has up to two positive roots (see below).

Inspecting the non-trivial equilibrium point, we must have $\bar{C} > 0$ in order to be biologically viable. The non-trivial equilibrium point P^* of the system of equations (3) is analyzed taking into account the relative values between γ and $n\beta$. For $\gamma > n\beta$, the condition $\bar{C} > 0$ is satisfied, from (13), when $\bar{B} < B_{\max}$, where

$$B_{\max} = \frac{\mu_C}{\gamma - n\beta}. \tag{15}$$

In this case, for $\bar{B} < B_{\max}$, we have $\bar{C} \geq C_0$, with $\bar{C} \to \infty$ when $\bar{B} \to B_{\max}$. Hence, $B_{\max}$ is the upper bound of bacteria concentration under the constraint of immune response. Notice that the polynomial $f\left(\bar{B}\right)$ assumes negative value at $B_{\max}$, that is, $f\left(B_{\max}\right) = -\left(\beta + \varepsilon\right)\lambda$. When the immune response deals appropriately with the mycobacterial infection, we have the situation $\gamma > n\beta$. The strong immune response is due to the combination of enhanced destruction of free bacilli and granulomas (ε and δ), high capacity of proliferation and recruitment of immune cells (γ), and decreased capacity of granuloma formation (β).

On the other hand, when $\gamma < n\beta$, from equation (13), we have $\bar{C} > 0$ for all values of $\bar{B} > 0$, because $B_{\max} < 0$, from equation (15). Additionally, comparing with equation (7), we have $\bar{C} \leq C_0$. We call as weak immune response when the granuloma formation (β) occurs more intensively than the proliferation of activated macrophages (γ), which may be due to high inoculation rate and/or delay in the immune system mounting appropriate response, or even due to immunedepression. The granuloma formation sequesters the macrophages, which diminishes the capacity of the immune response by lowering the amount of the defense cells. The phagocytized bacilli however proliferate inside the granuloma, which is unstable, especially in the lungs. For this reason when it reaches the lungs, it tends to liquefy and discharges bacteria through the bronchial tree, producing a tuberculous cavity and providing conditions in which bacterial population increases, the so called endogenous reactivation.[6]

To assess the local stability of the equilibrium point P^*, let us rewrite the coefficients of the characteristic polynomial (5) in terms of $\bar{B}$, by substituting $\bar{C}$ using equation (13), as

$$b_1 = \frac{g_1(\bar{B})}{h(\bar{B})} \quad and \quad b_0 = \frac{g_0(\bar{B})}{h(\bar{B})}, \tag{16}$$

where the auxiliary functions $g_1(\bar{B})$, $g_0(\bar{B})$ and $h(\bar{B})$ are

$$g_1(\bar{B}) = b_{12}\bar{B}^2 + b_{11}\bar{B} + b_{10}, \tag{17}$$

with coefficients $b_{12} = -\left(\gamma - n\beta\right)\left[\frac{\alpha}{k} - \left(\gamma - n\beta\right)\right]$, $b_{11} = \mu_C\left[\frac{\alpha}{k} - 2\left(\gamma - n\beta\right)\right]$ and $b_{10} = \mu_C^2$, and positive discriminant $\Delta_{g_1} = \left(\mu_C\frac{\alpha}{k}\right)^2$; the second function is

$$g_0(\bar{B}) = b_{02}\bar{B}^2 + b_{01}\bar{B} + b_{00}, \tag{18}$$

with coefficients $b_{02} = \left(\alpha - \mu_B\right)\left(\gamma - n\beta\right)^2$, $b_{01} = -2\mu_C\left(\gamma - n\beta\right)\left(\alpha - \alpha_0\right)$ and $b_{00} = \mu_C^2\left(\alpha - \alpha_0\right)$, and discriminant given by $\Delta_{g_0} =$

$-4\left[\mu_C\left(\gamma-n\beta\right)\right]^2\left(\beta+\varepsilon\right)\frac{\lambda}{\mu_C}\left(\alpha-\alpha_0\right)$; and the common denominator of b_1 and b_0 is given by

$$h(\bar{B})=\mu_C-\left(\gamma-n\beta\right)\bar{B}=\left(\gamma-n\beta\right)\left(B_{\max}-\bar{B}\right). \tag{19}$$

Therefore, if $b_1>0$ and $b_0>0$, then the equilibrium P^* is locally asymptotically stable. Nevertheless, the signals of b_1 and b_0 are defined by functions $g_1(\bar{B})$, $g_0(\bar{B})$ and $h(\bar{B})$.

Let us assess the equilibrium point $P=\left(\bar{B},\bar{C}\right)$ and the corresponding stability taking into account the relative values between γ and $n\beta$.

3.1. *Case $\gamma > n\beta$ – Strong immune response*

A strong immune response represents rapid proliferation and recruitment of macrophages in comparison with granuloma formation. Even in this situation, if the invading bacteria encounter favorable conditions ($\alpha>\alpha_0$), the infection can be maintained in the host, and the level of infection increases up to a limiting value (due to the carrying capacity k) as α increases. Since α_0 does not depend on γ, if the concentration of bacteria is settle at an infective level (the non-trivial equilibrium P^*, due to $\alpha>\alpha_0$), the rapid proliferation and recruitment of macrophages can only decrease the level of infecting population. The same conclusion remains valid with respect to the destruction of reactive granulomas by activated macrophages (δ).

When the immune system mounts a strong response against Mtb ($\gamma>n\beta$), the invading bacteria are eliminated independently of the initial inoculation if $\alpha\leq\alpha_0$, α_0 given by equation (8). To show this feature, we prove that P_0 is globally stable using the derivative of the Lyapunov function given in equation (10). When $\gamma>n\beta$, the invariant (biologically feasible) region is given by

$$\Omega_{>}=\left\{(B,C)\in R^2\mid B\leq B_0; C\geq C_0\right\},$$

where C_0 and B_0 are given by equations (7) and (12). Notice that $\dot{V}<0$ for $\alpha\leq\alpha_0$, and $\dot{V}=0$ if $B=0$. By inspecting the system of equations (3), we observe that the maximum invariant set is the trivial equilibrium point $P_0=(0,C_0)$. Hence, by the La-Salle Lyapunov Theorem,[12] P_0 is globally stable for $\alpha\leq\alpha_0$. When $\alpha>\alpha_0$, P_0 is unstable.

With respect to the non-trivial equilibrium point, positive solution of polynomial $f\left(\bar{B}\right)$, equation (14), we have two possibilities, according to the relative position between α and α_0, where α_0 is given by equation (8). On the other hand, the stability is achieved analyzing the signals of the

functions $g_1(\bar{B})$, $g_0(\bar{B})$ and $h(\bar{B})$. It is easy to show that the signals of $g_1(\bar{B})$ and $h(\bar{B})$ are positive for $\bar{B} < B_{\max}$ (not shown here), the range of biologically feasible, resulting in $b_1 > 0$. Hence, the stability of the equilibrium P^* which is biologically feasible is provided by the signal of b_0.

Case 1 – $\alpha < \alpha_0$. *Equilibrium*: We have one positive solution designed by B_p, and other is negative, B_n. However, the positive root is such that $\bar{B} = B_p > B_{\max}$, by the reason that $a_2 > 0$ and $f(B_{\max}) < 0$, which results in $\bar{C} < 0$ (biologically unfeasible). Hence, in this range we have only the trivial equilibrium point P_0, which is globally stable.

Stability: To show that $P^* = \left(\bar{B}, \bar{C}\right)$ is unstable, we split the interval in two sub-intervals.

Case 1.a – $\alpha < \mu_B$. The function $g_0(\bar{B})$, with $b_{02} < 0$, has two positive roots B^*_{01} and B^*_{02}, and, due to the fact that $g_0(B_{\max}) > 0$, we have $B^*_{01} < B_{\max} < B^*_{02}$, and consequently, $g_0(\bar{B}) > 0$, for $B_{\max} < \bar{B} < B^*_{02}$. Additionally, we have $h(\bar{B}) < 0$ for $\bar{B} > B_{\max}$. Then, $b_0 < 0$, for $B_{\max} < \bar{B} < B^*_{02}$, and the equilibrium $\bar{P}$ with coordinate B_p, since $B_p < B^*_{02}$, is unstable. Let us show that B_p is greater than $B_{\max}$ and smaller than B^*_{02}, when $\alpha < \mu_B$. If we show that $g_0(B_p) > 0$, than we guarantee that $B_p < B^*_{02}$. Let us define $B^* = (\gamma - n\beta) B_p > 0$. Then $g_0(B^*)$ can be written as

$$g_0(B^*) = (B^* - \mu_C)(\mu_B - \alpha)\left[-(B^* - \mu_C) + \frac{(\beta + \varepsilon)\lambda}{\mu_B - \alpha}\right] + (\beta + \varepsilon)\lambda B^*,$$

or, using $B^* - \mu_C = -\Phi + \sqrt{\Phi^2 + \Psi}/(2\alpha/k) > 0$, where $\Phi = \frac{\alpha}{k}\mu_C + (\mu_B - \alpha)(\gamma - n\beta)$ and $\Psi = 4\frac{\alpha}{k}(\gamma - n\beta)(\beta + \varepsilon)\lambda$ are positive values, then $g_0(B^*)$, changing only the terms between brackets, can be written as

$$g_0(B^*) = \frac{k(B^* - \mu_C)(\mu_B - \alpha)}{2\alpha}\left[\Phi + \Omega - \sqrt{\Phi^2 + \Psi}\right] + (\beta + \varepsilon)\lambda B^*,$$

where $\Omega = \frac{2\alpha(\beta+\varepsilon)\lambda}{k(\mu_B - \alpha)}$. Since $\alpha < \mu_B$, we have $g_0(B^*) > 0$ if $\Phi + \Omega - \sqrt{\Phi^2 + \Psi} > 0$. This inequality is true whenever $A = (\Phi + \Omega)^2 - \left(\sqrt{\Phi^2 + \Psi}\right)^2 > 0$, where

$$A = 4(\beta + \varepsilon)\lambda\left[\frac{\alpha}{k(\mu_B - \alpha)}\right]^2 \left[(\beta + \varepsilon)\lambda + \mu_C(\mu_B - \alpha)\right].$$

Being $A > 0$, we have $g_0(B_p) > 0$, and we conclude that $B_{\max} < B_p < B^*_{02}$.

Case 1.b – $\mu_B < \alpha < \alpha_0$. The function $g_0(\bar{B})$, with $b_{02} > 0$, has one negative solution B^*_{0n} and a positive, B^*_{0p}, and due to the fact that $g_0(B_{\max}) > 0$, we have $B^*_{0p} < B_{\max}$. Hence, $g_0(\bar{B}) > 0$, for $\bar{B} > B_{\max}$,

and $b_0 < 0$, due to $h(\bar{B}) < 0$ in this interval. The non-trivial equilibrium, biologically unfeasible, is unstable.

Case 2 – $\alpha > \alpha_0$. *Equilibrium*: We have two positive solutions designed by B_p^- and B_p^+ for, respectively, small and big roots. However, the positive roots are such that $B_p^- < B_{\max} < B_p^+$, by the reason that $a_2 > 0$ and $f(B_{\max}) < 0$. However, for $\bar{B} = B_p^+ > B_{\max}$, we have $\bar{C} < 0$, which is not biologically feasible. Hence, we have a unique non-trivial equilibrium value given by $\bar{B} = B_p^-$, besides the trivial equilibrium point P_0, which is unstable.

Stability: Notice that the function $g_0(\bar{B})$, with $b_{02} > 0$ and $b_{00} > 0$, does not have real roots, due to $\Delta_{b_0} < 0$, resulting in $g_0(\bar{B}) > 0$, for all $\bar{B}$. Additionally, being $h(\bar{B}) > 0$, for $\bar{B} < B_{\max}$, and otherwise, $h(\bar{B}) < 0$, the biologically feasible equilibrium point ($B_p^- < B_{\max}$) is LAS, while the big root $B_p^+ > B_{\max}$ is unstable.

According to the model, what mimics strong immune response is the increase in the number of macrophages in comparison with the level found before the infection, that is, $\bar{C} \geq C_0$. This result comes out due to the proliferation of activated and recruitment of naive macrophages surpassing the capacity of granuloma formation by the naive macrophages, showing that the immune system responds very well to deal with the invading Mtb. Moreover, when the granulomas formed by infected macrophages reach low density at the steady state, a good prognostic with respect to future reactivation of TB can be done.

The concentration of Mtb at the steady state $\bar{B}$ corresponds to the small positive root B_p^- (lower than $B_{\max}$) of the polynomial $f\left(\bar{B}\right)$, equation (14), which is LAS for $\alpha > \alpha_0$. Another positive solution B_p^+ for $f\left(\bar{B}\right)$ is such that $B_p^+ > B_{\max}$, resulting in biologically unfeasible $\bar{C} < 0$. Asymptotically, we have $\lim_{\alpha\to\infty} B_p^- = k$ ($\lim_{\alpha\to\infty} B_p^+ = B_{\max}$), for $B_{\max} > k$; otherwise, $\lim_{\alpha\to\infty} B_p^- = B_{\max}$ ($\lim_{\alpha\to\infty} B_p^+ = k$).

Summarizing, for $\gamma > n\beta$, the trivial equilibrium P_0 is globally stable for $\alpha \leq \alpha_0$, while the non-trivial equilibrium P^*, with $\bar{B} = B_p^-$, is LAS for $\alpha > \alpha_0$. Due to the discriminant Δ_Λ calculated from characteristic equation (5) and $b_1 > 0$, P^* is either stable node or focus. Therefore, forward bifurcation occurs at $\alpha = \alpha_0$.

3.2. *Case $\gamma < n\beta$ – Weak immune response*

A weak immune response is characterized by a slow proliferation and recruitment of macrophages in comparison with granuloma formation.

When the immune system mounts a weak response against Mtb ($\gamma < n\beta$), the invading bacteria are eliminated independently of the initial inoculation if $\alpha \leq \mu_B$. This feature can be shown proving that P_0 is globally stable using the derivative of the Lyapunov function given in equation (9). When $\gamma < n\beta$, the invariant (biologically feasible) region is given by

$$\Omega_< = \left\{(B, C) \in R^2 \mid B \leq B_0; C \leq C_0\right\},$$

where C_0 and B_0 are given by equations (7) and (12). In this case, we observe that $\dot{V} < 0$ for $\alpha \leq \mu_B$, and $\dot{V} = 0$ if $B = 0$. By inspecting the system of equations (3), we observe that the maximum invariant set is the trivial equilibrium point P_0. Hence, by the La-Salle Lyapunov Theorem,[12] P_0 is globally stable for $\alpha \leq \mu_B$.

With respect to the non-trivial equilibrium point P^*, we have up to two points corresponding to the positive roots of the polynomial $f\left(\bar{B}\right)$, equation (14). The non-trivial equilibrium values are studied considering μ_B and α_0. With respect to the local stability, when $\gamma < n\beta$, we have $h(\bar{B}) > 0$ for all values of $\bar{B} > 0$. On the other hand, the function $g_1(\bar{B})$ has two negative values $B^*_{11} = -\mu_C / (n\beta - \gamma)$ and $B^*_{12n} = -\mu_C / \left(n\beta - \gamma + \frac{\alpha}{k}\right)$, besides the fact that $b_{12} > 0$. Hence, we have $g_1(\bar{B}) > 0$, for all values of $\bar{B} > 0$. Then, we have $b_1 > 0$ for all positive $\bar{B}$, leaving the stability of the equilibrium points being determined only by the signal of $g_0(\bar{B})$, which provides signal of b_0.

Case 1 – $\alpha < \mu_B$. *Equilibrium*: In this case, all coefficients a_2, a_1 and a_0 of $f\left(\bar{B}\right)$ are negative. Hence, there is not non-trivial equilibrium point.

Stability: The function $g_0(\bar{B})$ has all coefficients b_{02}, b_{01} and b_{00} negatives, which implies that $g_0(\bar{B}) < 0$ for all $\bar{B} > 0$. Hence, we have $b_0 < 0$. As we have shown, we do not have positive solutions for $\alpha < \mu_B$, and we have shown also that the trivial equilibrium P_0 is globally stable in this range.

Case 2 – $\mu_B < \alpha < \alpha_0$. In this case the interval was split in two sub-intervals, taking into account $\bar{\alpha}$ which is the big positive solution of

$$\left[1 + \left(\frac{\mu_C}{k(n\beta-\gamma)}\right)^2 + \frac{2\mu_C}{k(n\beta-\gamma)}\right]\alpha^2 - 2\left[\mu_B + \frac{\mu_C\mu_B}{k(n\beta-\gamma)} + \frac{2(\beta+\varepsilon)\lambda}{k(n\beta-\gamma)}\right]\alpha + \mu_B^2 = 0. \tag{20}$$

Case 2.a – $\mu_B < \alpha < \bar{\alpha}$. *Equilibrium and stability*: Idem to **case 1**.

Case 2.b – $\bar{\alpha} < \alpha < \alpha_0$. In this case, we have two possibilities, according to $\bar{k}$ given by

$$\bar{k} = \frac{\mu_C}{n\beta - \gamma}\left[1 + \frac{\mu_C\mu_B}{(\beta+\varepsilon)\lambda}\right]. \tag{21}$$

Case 2.b.1 – $k < \bar{k}$. *Equilibrium and stability*: Idem to **case 1**.

Case 2.b.2 – $k > \bar{k}$. *Equilibrium*: We have two positive solutions for $f\left(\bar{B}\right)$, given by equation (14), designed as B_p^s and B_p^g for, respectively, small and big roots. At $\alpha = \bar{\alpha}$, we have $B_p^s = B_p^g$, and as α increases, B_p^g increases and B_p^s decreases, assuming zero value at $\alpha = \alpha_0$. When the carrying capacity k is higher than its critical value $\bar{k}$, we have two non-trivial (and biologically viable) equilibrium points.

Stability: The function $g_0(\bar{B})$, with $b_{02} > 0$, has one negative solution B_{0n}^* and a positive root B_{0p}^*, and due to the fact that $g_0(B_{\max}) > 0$, we have $B_{0p}^* < B_{\max}$. Hence, $g_0(\bar{B}) < 0$, for $0 < \bar{B} < B_{0p}^*$, $g_0(\bar{B}) > 0$, for $B_{0p}^* < \bar{B} < B_{\max}$, and $g_0(\bar{B}) > 0$, for $\bar{B} > B_{\max}$. In order to prove that the small positive solution B_p^s is unstable, while the big solution B_p^g is LAS, we must show that $B_p^s < B_{0p}^* < B_p^g$. Let us define $\bar{f}\left(\bar{B}\right) = -f\left(\bar{B}\right)$. Being B_p^s and B_p^g the positive roots of $\bar{f}\left(\bar{B}\right)$, if we show that $\bar{f}\left(B_{0p}^*\right) < 0$, then $B_p^s < B_{0p}^* < B_p^g$. First, using the fact that $g_0\left(B_{0p}^*\right) = 0$, where B_{0p}^*, given by

$$B_{0p}^* = \frac{\mu_C}{(\alpha - \mu_B)(n\beta - \gamma)}\left[(\alpha_0 - \alpha) + \sqrt{(\alpha_0 - \alpha)(\beta + \varepsilon)\frac{\lambda}{\mu_C}}\right],$$

is positive for $n\beta > \gamma$ and $\mu_B < \alpha < \alpha_0$, we can write $\bar{f}\left(B_{0p}^*\right)$ as $\bar{f}\left(B_{0p}^*\right) = \frac{(\beta+\varepsilon)\lambda}{\mu_C(\alpha-\mu_B)} B_{0p}^* F\left(B_{0p}^*\right)$, with

$$F\left(B_{0p}^*\right) = -\left[(\alpha - \mu_B)(n\beta - \gamma) - \mu_C \tfrac{\alpha}{k}\right] + \left[(\alpha - \mu_B)(n\beta - \gamma) + \mu_C \tfrac{\alpha}{k}\right]\sqrt{\tfrac{\mu_C(\alpha_0 - \alpha)}{(\beta+\varepsilon)\lambda}}, \tag{22}$$

where the proportionality between $F\left(B_{0p}^*\right)$ and $\bar{f}\left(B_{0p}^*\right)$ is positive for $n\beta > \gamma$ and $\mu_B < \alpha < \alpha_0$. Hence, whenever $F\left(B_{0p}^*\right) < 0$, we have $\bar{f}\left(B_{0p}^*\right) < 0$. Second, in order to show that $F\left(B_{0p}^*\right) < 0$, we must show that the absolute value of the first term in the second member of equation (22) is higher than the second term, or, the difference between squared values of the first term and the second term must be positive. From this inequality, we can obtain an equation $G^*(k^*)$, given by $G^*(k^*) = \frac{\mu_C(\alpha-\mu_B)}{(\beta+\varepsilon)\lambda} G(k^*)$, with $k^* = (n\beta - \gamma)k$, where

$$G(k^*) = \left[(\alpha - \mu_B)k^* - \mu_C\alpha\right]^2 - 4\mu_C\alpha(\alpha_0 - \alpha)k^*,$$

such that when $G(k^*) > 0$, we have $F\left(B_{0p}^*\right) < 0$. The second order polynomial $G(k^*)$ has two positive solutions, named G_s and G_g, such that $0 < G_s < G_* < G_g$, where $G_* = \frac{\mu_C\alpha}{\alpha - \mu_B}$, which decreases from ∞ (at $\alpha = \mu_B$) to $\bar{k}^* = (n\beta - \gamma)\bar{k}$ (at $\alpha = \alpha_0$), where $\bar{k}$ is given by equation (21). Clearly we have $G(k^*) > 0$ for $k^* > G_g$. When

$\alpha = \mu_B$, we have $G(k^*) = (\mu_C\mu_B)^2 - 4\mu_B(\beta+\varepsilon)\lambda k^*$, resulting in $G(\bar{k}^*) = -\left[3(\mu_C\mu_B)^2 + 4\mu_C\mu_B(\beta+\varepsilon)\lambda\right] < 0$; hence $G(k^*) < 0$, for $k^* > \bar{k}^*$. On the other hand, when $\alpha = \alpha_0$, we have $G(k^*) = \left[\frac{(\beta+\varepsilon)\lambda}{\mu_C}k^* - \mu_C\alpha_0\right]^2$, resulting in $G(\bar{k}^*) = 0$; hence $G(k^*) > 0$, for $k^* > \bar{k}^*$. Therefore, being $G(k^*)$ a continuous function, there is an α^*, with $\mu_B < \alpha^* < \alpha_0$, such that $G(k^*) > 0$, when $k^* > G_g > \bar{k}^*$. Note that $G_* > \bar{k}^* = (n\beta - \gamma)\bar{k}$ for $\mu_B < \alpha < \alpha_0$. Therefore, when $\alpha^* < \alpha < \alpha_0$ and $k^* > \bar{k}^*$, we have $G(k^*) > 0$, that is, $B_p^s < B_{0p}^* < B_p^g$. Remember that the existence of two positive solutions requires $k > \bar{k}$ and $\mu_B < \bar{\alpha} < \alpha < \alpha_0$, where $\bar{\alpha}$ is given by the big positive solution of equation (20).

Case 3 – $\alpha > \alpha_0$. *Equilibrium*: We have one positive solution B_p, and one negative root, B_n, because $a_2 < 0$ and $a_0 > 0$. Hence, we have only one biologically feasible solution B_p. In this case we have one non-trivial equilibrium point. Note that B_p is extension of B_p^g as α surpasses α_0, while B_n (negative root) is extension of B_p^s, which assumes zero value at $\alpha = \alpha_0$, or $B_p^s(\alpha_0) = 0$. Asymptotically, we have $\lim_{\alpha\to\infty} B_p = k$ and $\lim_{\alpha\to\infty} B_n = B_{\max} < 0$.

Stability: The function $g_0(\bar{B})$ has all coefficients b_{02}, b_{01} and b_{00} positive, and $\Delta_{b_0} < 0$, resulting in $g_0(\bar{B}) > 0$, for all $\bar{B}$. Hence, the biologically feasible equilibrium point B_p is LAS.

What typically mimics weak immune response is $\bar{C} \leq C_0$ for all values of $\bar{B}$. When the proliferation and recruitment of macrophages is slow and granuloma formation is relatively rapid, the immune system does not respond so well to deal with the invading Mtb. Moreover, granulomas formed by infected macrophages reach considerable density at the steady state, which can facilitate a future reactivation of TB. Another interesting feature occurs when the carrying capacity is increased: If the initial inoculation of Mtb is high, then the infection can be settle at a persistent level even so the bacterium has low capacity to replicate ($\alpha < \alpha_0$).

Summarizing, for $\gamma < n\beta$, we have two possibilities. For lower carrying capacity, $k < \bar{k}$, $\bar{k}$ given by equation (21), we have forward bifurcation like the previous case (strong immune response, $\gamma > n\beta$): the trivial equilibrium P_0 is LAS for $\alpha \leq \alpha_0$, while the non-trivial equilibrium P^*, with $\bar{B} = B_p$, is LAS for $\alpha > \alpha_0$. Bifurcation occurs at $\alpha = \alpha_0$. However, for $k > \bar{k}$, when $\bar{\alpha} < \alpha < \alpha_0$, $\bar{\alpha}$ given by equation (20), we have two positive equilibrium values, B_p^s (unstable, saddle point) and B_p^g (LAS, stable node). At $\alpha = \bar{\alpha}$ occurs backward bifurcation, and the unstable branch of solutions B_p^s forms

the break point.[13] In both cases, the trivial equilibrium P_0 is globally stable for $\alpha \leq \mu_B$, and P^* is stable node (due to $\Delta_\Lambda > 0$ and $b_1 > 0$ from equation (5)).

4. Numerical results

Let us illustrate our findings by drawing the bifurcation diagrams. The values of the model's parameters are: $\alpha = 5 \times 10^{-3}$, $\mu_B = 1 \times 10^{-3}$, $\mu_C = 1 \times 10^{-2}$, $\mu_G = 1 \times 10^{-3}$, $\mu_T = 4 \times 10^{-4}$, $\theta = 3 \times 10^{-3}$ (all in $days^{-1}$); $\delta = 1.25 \times 10^{-7}$, $\gamma = 1.0 \times 10^{-5}$, $\beta = 1.25 \times 10^{-8}$, $\varepsilon = 1.25 \times 10^{-7}$, (all in $[\bullet]^{-1} \times days^{-1}$, where $[\bullet]$ is concentration of the variables), $\lambda = 50$ (in $[M] \times days^{-1}$), $k = 1.0 \times 10^4$ and $n^{-1} = 2$. These values results in $\alpha_0 = 1.69 \times 10^{-3}\ days^{-1}$. Hereafter we will omit units.

In Figure 1a we show the forward bifurcation diagram ($\bar{B} \times \alpha$) using the values given above. The bifurcation occurs at $\alpha = \alpha_0$, and the concentration of free bacteria B_p^- reaches the asymptote $B_{\max}$. Note that at $\alpha = 0.005$ the asymptote is practically achieved. In Figure 1b we show the backward bifurcation diagram ($\bar{B} \times \alpha$). In order to do this, we consider the values given above, except $k = 5.0 \times 10^7$ and $\gamma = 1.0 \times 10^{-9}$. In this case α_0 is the same obtained above, and we have $\bar{\alpha} = 2.36 \times 10^{-5}$, $n\beta - \gamma = 5.29 \times 10^{-9}$, $\bar{k} = 4.68 \times 10^6$ and $\bar{\alpha} = 1.32 \times 10^{-3}$. The backward bifurcation occurs at $\alpha = \bar{\alpha}$ due to the fact that $k > \bar{k}$ and at $\alpha = \alpha_0$ the decreasing branch assumes zero value.

In order to illustrate the role of the break point, which separates two basins of attraction, we show the dynamical trajectories of B, C, G and T using the values given above, except $\gamma = 1.0 \times 10^{-9}$, $\alpha = 1.40176 \times 10^{-2}$ and $k = 5.0 \times 10^7$. The assigned values for α and k are such that $\bar{\alpha} < \alpha < \alpha_0$ and $k > \bar{k}$. In Figure 2a, we supply $B(0) = 0.99 \times B_p^s$, $C(0) = \bar{C}(B_p^s)$, $G(0) = \bar{G}(B_p^s)$ and $T(0) = \bar{T}(B_p^s)$ as the initial conditions, where B_p^s is the small positive solution of the equation (14), and $\bar{G}$, $\bar{T}$ and $\bar{C}$ are the equilibrium values evaluated at $\bar{B} = B_p^s$, using equations (4) and (13). Being the initial conditions below the unstable branch (the break point), the trajectories go to the trivial equilibrium point. In Figure 2b we supply $B(0) = 1.01 \times B_p^s$, $C(0) = \bar{C}(B_p^s)$, $G(0) = \bar{G}(B_p^s)$ and $T(0) = \bar{T}(B_p^s)$, above the unstable branch. The dynamical trajectories go to the non-trivial equilibrium obtained with big positive root of equation (14), B_p^g. In both cases we have stable nodes.

Figure 2a shows the immune response clearing the infecting Mtb, and C reaches the asymptote C_0. Nevertheless, Figure 2b shows that, even in

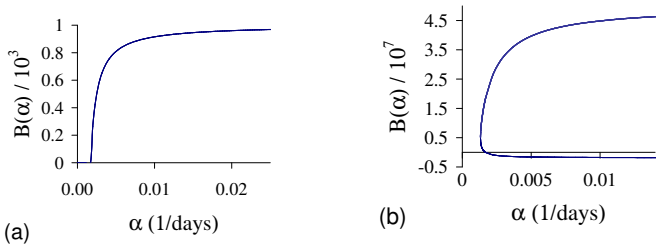

Figure 1. The curve illustrates the forward bifurcation (cases $\gamma > n\beta$; and $\gamma < n\beta$ and $k < \bar{k}$) occurring at $\alpha = \alpha_0$, and due to $B_{\max} < k$, the asymptote reaches $B_{\max}$ (a). The curve shows the backward bifurcation (case $\gamma < n\beta$ and $k > \bar{k}$) occurring at $\alpha = \bar{\alpha}$ (b). The asymptote reaches k, because $B_{\max} < 0$. The thick curve is the stable equilibrium point, forming the increasing branch, while the thin curve corresponds to unstable (break point) equilibrium point, which assumes zero value at $\alpha = \alpha_0$, and negative values since after. The scale in the vertical axis must be multiplied by the factor shown in the denominator of the variable.

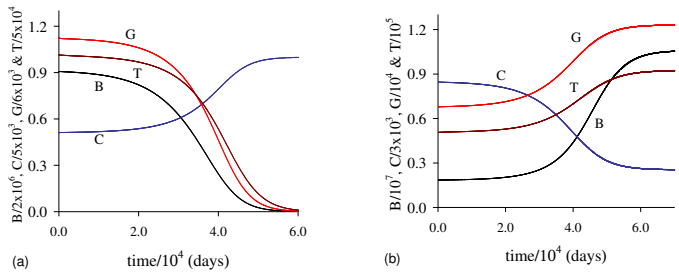

Figure 2. We show the dynamical trajectories B, C, G and T. The backward bifurcation generates break point (unstable branch), and the trajectories depend on the initial conditions supplied to the dynamical system. When the initial conditions are situated below the unstable branch, the trajectories go to the trivial equilibrium point (a); and the dynamical trajectories go to the stable non-trivial equilibrium because the initial conditions are situated above the unstable branch (b). The scale in the vertical and horizontal axes must be multiplied by the factor shown in the denominator of the variables.

the case $\alpha < \alpha_0$, if the level of inoculation of bacteria is sufficiently higher (above the breaking point) and, additionally, they encounter favorable envi-

ronment ($k > \bar{k}$), bacteria population can be established in the host. In this case, the dynamics of interaction of bacteria with immune response occurs more slowly (10 times) than the case of strong immune response. Moreover, the level of reactive granulomas reaches higher concentration (main reason is due to $\gamma < n\beta$) showing a more predisposition for future reactivation of TB.[6]

Let us consider tobacco smokers. Epidemiological evidence indicates that smoking is associated with risk of being infected with Mtb, risk of developing TB, risk of developing more severe TB and risk of dying of TB.[14] The mechanism of increased susceptibility to infections, as Mtb, in smokers is multifactorial and includes alteration of the structural and immunologic host defenses.[15] First, mechanical and structural changes include peribronchiolar inflammation and fibrosis, increased mucosal permeability, impairment of the mucociliary clearance, changes in pathogen adherence and disruption of the respiratory epithelium. With respect to immunologic mechanisms, the effects are various: Elevated peripheral white blood cells count (30% higher among smokers than nonsmokers), marked decrease in the percentage and absolute number of CD4+ cells and increase in CD8+ with lower ratio CD4+/CD8+, deficit in cell-mediated immunity in the lung alveolus, significant increases in the percentage of macrophages (having a greater inhibitory effect on lymphocyte proliferation, which shows an immunosuppressive effect) in bronchoalveolar lavage fluid, and impairment of natural killer (NK) cell activity.

Quantitatively, the surface of alveolar area producing tuberculous cavity (k) and granuloma formation (β) increase among tobacco smokers due to decrease in immune response, mechanical disruption of cilia function results in a weak barrier against invading bacteria (α increases, and/or the initial inoculation increases), defects in macrophage immune response increase the non-activated alveolar macrophages (increasing $\frac{\lambda}{\mu_C}$ in conjunction with β increases the formation of granulomas), and/or CD4+ lymphopenia occurs (ε and γ decrease). As a consequence, we have a decreasing in α_0, an increasing in the number of invading (inoculation of) Mtb, and possibly in $\gamma < n\beta$, which may allow the occurrence of backward bifurcation. Hence, all these factors in combination can result in an increased TB risk.[15]

In a well responding immune system, CD4+ cells are efficiently activated, and are driven to proliferate rapidly. However, in smokers, it is observed deficit in cell-mediated immunity in the lung alveolus and significant increase in the percentage of macrophages having a greater inhibitory effect on lymphocyte proliferation. In other words, we have the reduction

in the capacity of activating lymphocytes (q) and the decrease in the proliferation (γ) of these activated cells. Hence, the phagocytosis and further lysis of bacteria (ε) and the destruction of granulomas (δ) by activated macrophages are seriously impaired. Conversely, the granuloma formation (β) is increased in a weak cell-mediated immune response due to phagocytosis of bacteria by naive macrophages, and mechanical disruptions increase k. As a consequence, the risk of direct progression to TB is increased.

5. Conclusion

A simple model was proposed to describe the progression of Mtb infection. In this work, the continuously varying action of cytokines and other immune cells (as dendritic cells, CD4+ and Cd8+ T cells, etc.) was approximated by a constantly acting parameter q.

A simple model was applied to explain the direct progression to TB. The analysis of the model showed the existence of a threshold parameter α_0, which is a key parameter to control the infection. If the intrinsic growth rate of mycobacteria is lower than this value, the infection does not fade out and occurs the persistence of the bacilli. To control the infection, the parameter α_0 must be increased in order to surpass α. One of the controlling mechanisms is the administration of antibiotics in order to increase μ_B. A vaccine with the property of increasing the activation and proliferation of macrophages (described by the parameters ε and δ) should be a potent prophylactic control, since the Mtb infection requires cellular response mounted by human immune system to be controlled. Note that the phagocytosis of bacteria by naive macrophages (described by the parameter β) also reduces the capacity of mycobacterial proliferation. The improvement of the nutrition factor due to socioeconomic conditions getting better influences positively the health conditions of the population. As a consequence, the immune system is enhanced, by increasing the alveolar macrophages (parameter $C_0 = \lambda/\mu_C$).

The rate of proliferation and recruitment of macrophages (γ) plays an important role. This parameter does not contribute to the threshold value α_0, because its influence occurs moments after the infection. This overall parameter is combination of γ_M and r_M according to $\gamma = q\,(\gamma_M + r_M)$. Obviously, without the cell-mediated response by action of activated lymphocytes ($q = 0$), there is not recruitment of naive macrophages neither the proliferation of activated macrophages. An interesting property is regarding to the strength of immune response: If recruitment is more intense than

proliferation, there is more recruitment of naive macrophages in comparison with proliferation of activated macrophages, even in a strong immune response. This fact can compromise the efficiency of cell-mediated immune response against Mtb infection: Increasing naive macrophages, the granuloma formation can be enhanced. Hence, in the case of Mtb infection, proliferation of activated macrophages must prevail over recruitment of naive macrophages, attesting the very importance of CD4+ cells (and dendritic cells) to circumscribe or to eliminate invading bacteria.

We applied the results obtained from the model with respect to enhanced and weakened immune responses, taking smokers as an example. In tobacco smoking individual, for instance due to mechanical disruption of cilia function,[16] the alveolar regions where invading bacteria can reach could be increased, which is described quantitatively by increasing the carrying capacity k. The immune response is impaired by decreasing CD4+ cells (q is decreased, decreasing ε, δ and γ, and increasing β)[17−18] and the number of alveolar macrophages can also be increased (increasing C_0),[19] but acting as immunosuppressive way.[20] Moreover, the impairment of NK cell activity is a potential mechanism for the increased incidence of infection among smokers.[21] As a consequence, when an elevate quantity of bacteria infects persons with weakened immune response (especially in the case of $\gamma < n\beta$ and $\alpha < a_0$), granulomas are formed in higher quantity. Therefore, there is an increase in the success of the invading mycobacteria being persistent ($\alpha > \bar{\alpha}$), or, even in the case of clearance of invading bacteria, the solid caseous material derived from reactive granulomas are left in elevate concentration in the host. This fact shows that the reactivation of TB can be facilitated and also can occur in a short period of time after prime infection. Reactivation of TB is dealt with in a companion paper.[6]

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