Macrophage-Activating and Tissue-Damaging Immune Responses to *M. tuberculosis*

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Abstract

The two principal immune responses against *Mycobacterium tuberculosis* are the macrophage-activating response (MAR) and the tissue-damaging response (TDR). In the TDR, T-lymphocytes kill those macrophages that permit uncontrolled bacillary growth in their cytoplasm, this mechanism is believed to be responsible for the caseous necrosis in the host's lung tissue. In the MAR, macrophages are activated by T-lymphocytes in order to digest the bacilli as they are engulfed. With the use of a mathematical model we intend to find a proper interplay of both mechanisms to obtain an effective immune response where tissue damage is minimized and still the disease is eradicated.

1 Introduction

Tuberculosis is the leading cause of death in the world –3 million deaths per year– from an infectious disease. It has been estimated that one third of the human population, or about 1.7 billion people, is infected with *Mycobacterium tuberculosis*, the causative agent of TB. Furthermore, the global re-emergence of TB has been partially linked to the HIV epidemic. A compromise HIV infected immune system often leads either to the reactivation of dormant tuberculous infections or to an increase in susceptibility to new TB infections. Cure rates of about 85% for active TB disease have been achieved but multidrug-resistant strains of the bacillum have emerged as a result of lack of compliance with treatment protocols. The emergence of antibiotic resistant TB-strains is a major health issue in the world.

There are a number of factors that determine the clinical manifestation of tuberculosis, some related to the host and others to the bacillum. Factors involving the host may include its immune status, the presence of additional diseases, and its history of immunization. Factors involving the bacillum include the virulence of the organism and its preference for specific tissues. It is estimated that among tuberculin-positive persons, there is a 10% likelihood of developing active tuberculosis with the rest remaining as a latent (asymptomatic infections). Even though the immunology of tuberculosis is well understood today, many important questions have yet to be answered concerning the interplay among mechanisms that may result in the various clinical manifestations. It is known that T-lymphocytes responding to antigenic mediators (interleukins 1 and 2, gamma interferon, tumor necrosis factor alpha) are the major players of the immune response to *M. tuberculosis*. The specific interaction of these mediators is a major topic of current research (Chan et al., 1994; Barnes et al., 1994; Ivanyi et al., 1994; Dannenberg et al., 1994; Rook et al., 1994). In this work, we address this specific interaction using a simple mathematical model.

2 Biological Background

The first response of the immune system to *Mycobacterium tuberculosis* is primarily carried out by mononuclear phagocytes (macrophages) and T-lymphocytes (T-cells). At the primary infection site (alveolar sacks) macrophages ingest the bacilli, triggering an immunological battle where an interplay between a macrophage-activating response (MAR) and a tissue-damaging response (TDR) will determine the outcome of the infection. Research about the pathogenesis of tuberculosis has identified five main stages of the immunological response (Dannenberg, 1994): (1) onset; (2) symbiosis between macrophages and bacilli; (3) caseous necrosis formation stage produced by the TDR; (4) interplay between MAR and TDR; and (5) liquefaction of caseous tissue. The course that the disease will take depend on the balance between TDR and MAR in the interplay stage.

The first stage begins with the inhalation of the tuberculosis bacillum into the alveolar region. The existence of partially activated macrophages is due to the casual inhalation of foreign particles that maintain these cells in a nonspecific alert state. Nonspecifically activated macrophages in the alveoli ingest the bacilli entering the body. If these macrophages are sufficiently microbicidal, with respect to the virulence of the bacilli, they digest the engulfed bacteria, and no disease will occur. If these macrophages are unable to kill the bacilli and if they permit intracellular bacterial growth then, they will explode because of the high bacillary load. This explosion triggers the second stage of the infection.

In the second stage, nonactivated macrophages from the bloodstream are attracted to the explosion site by specific chemotactic factors including the debris products of the bacilli and the exploding macrophages. Since these macrophages are not activated, they cannot inhibit or digest the bacilli. Neither can the bacteria cause any damage to these macrophages because they have not developed tuberculin-type hypersensitivity. Non activated macrophage engulf released bacteria and enter into a symbiotic relationship with the bacilli. Additional macrophages and bacilli accumulate around the initial lesion with the bacilli experiencing logarithmic growth inside the macrophages. This symbiotic stage lasts approximately 7 to 21 days after the initial infection.

The third stage of the disease begins when intracellular bacterial growth stops; the host becomes tuberculin positive, and the caseous necrotic center enlarges around the initial lesion. T-cells entering the infected region destroy those macrophages that cannot control the growth of the bacteria inside of them thus eliminating the favorable intracellular environment of the macrophages' cytoplasm. In this caseous medium, bacteria cannot multiply because of the unfavorable chemical conditions. However, they can live in it for years in a dormant, non-metabolic state. The growth of the necrotic center and the initial activation of macrophages by the T-cells are the result of this third stage.

A large amount of antigen around the necrotic center triggers the production of lymphokines, attracting lymphocytes and activated macrophages. These events mark the start of the fourth stage of the infection, that is, the interplay between TDR and MAR. The length of time that it takes the immune system to increase the number of activated macrophages will be a key factor for the survival of the host. If the number of activated macrophage is not increased, then tissue-damaging responses predominate, the host will lose a dangerously high amount of tissue and the disease will progress.

Liquified lesions are an excellent medium for bacillary growth. Also, these lesions are saturated with antigenic products that are toxic to the host's tissue and to phagocytic cells. Therefore, the bacteria multiply in a favorable extracellular medium, making it possible for them to reach other parts of the body, and diminishing the possibilities of an effective immune response. Hence, even if macrophage activation is strong, progression of the disease may still occur via the eventual liquefaction of lesions.

How can the interaction of T-cells and macrophages be controlled to mantain an effective and favorable interplay between the activation of macrophages and the destruction of tissue? How can the liquefaction process disrupt an otherwise strong and sufficient immune response where the growth of bacilli is effectively stopped? In this study we propose a mathematical model for the immune response to *M. tuberculosis*. The model is proposed in order to answer questions about the immune mechanisms against tuberculosis. A model for the immune

response to tuberculosis and HIV is presented in Kirschner. (pre-print). This work does not explore the specific mechanisms modeled in our work, namely, the tissue-damaging and the macrophage-activating responses, and the liquefaction process. Here, we consider the key cell populations, the pathogen and the tissue damage associated with the immune response. In Section 3 we present the model along with its general underlying assumptions. In Section 4 we analyze this system of nonlinear differential equations and discuss our numerical results. Section 5 sumarizes our findings, suggest possible answers to the questions raised above.

3 The Model

Our model consists of five ordinary differential equations which model the rate of change with respect to time of key factor associated with the immune responses. Uninfected macrophages at time t are represented by $M(t)$; infected macrophages are represented by $M_i(t)$; $M_c(t)$ represents the population of cells experiencing necrotic tissue or tissue damage; the population of T-lymphocytes is given by $T(t)$; and *M. tuberculosis* is represented as $B(t)$. We assume that disease dynamics occur in the alveolar region of the lungs. The model is given by the following system of nonlinear differential equations:

$$\frac{dM}{dt} = \beta - \epsilon MB + \alpha TM_i - \mu M \tag{1}$$

$$\frac{dM_i}{dt} = \epsilon MB - (\alpha + \eta)TM_i - \mu M_i \tag{2}$$

$$\frac{dM_c}{dt} = \eta TM_i - \lambda M_c \tag{3}$$

$$\frac{dT}{dt} = \Lambda + rT\frac{M_i}{a + M_i} - \delta T \tag{4}$$

$$\frac{dB}{dt} = \rho B(K - B) - \epsilon MB + \mu SM_i + \lambda LM_c \tag{5}$$

Equation (1) is the rate of change in the uninfected macrophage population where β is the recruitment rate of new uninfected macrophages which enter the alveolar region at a constant rate from the bloodstream. The term $-\epsilon MB$ represents the engulfment of bacteria by macrophages while αTM_i gives the rate of return of recovered macrophages. This recovery process is activated T-cells. The term μM is the natural death of these macrophages.

Equation (2) is the rate of change in the number of infected macrophages. Macrophages

become infected when they engulf or ingest bacteria. Infected macrophages are either activated or necrotized by T-cells, $(\alpha+\eta)TM_i$. Necrotized macrophages pass to the M_c class. The term μM_i is the natural death of infected M cells.

Equation (3) gives the rate at which tissue becomes damaged by the necrotizing process. Caseous tissue may liquefy and this possibility is represented by λM_c.

Equation (4) models the rate of change in the T-cell population. The first term denotes the recruitment rate (assumed constant) from the source organs (thymus, bone marrow). The second term gives the replication rate of T-cells in the presence of infected presenter cells while the third term is the natural death rate of these lymphocytes.

Equation (5) models the rate of change of the bacilli population. Bacilli have an assumed logistic growth rate; the loss of free bacteria is due to their engulfment by macrophages; the release of bacteria is the death of infected macrophages; the source of bacilli is liquified caseous tissue.

4 Mathematical and Numerical Analysis

4.1 Disease-Free Equilibrium

We begin the analysis by calculating the Jacobian matrix for this system of equations:

$$J(x) = \begin{pmatrix} -\epsilon B - \mu & \alpha T & 0 & \alpha M_i & -\epsilon M \\ \epsilon B & -(\alpha+\eta)T - \mu & 0 & -(\alpha+\eta)M_i & \epsilon M \\ 0 & \eta T & -\lambda & \eta M_i & 0 \\ 0 & rT\frac{a}{(a+M_i)^2} & 0 & r\frac{M_i}{a+M_i} - \delta & 0 \\ -\epsilon B & \mu S & \lambda L & 0 & \rho K - 2\rho B - \epsilon M \end{pmatrix}$$

The disease-free equilibrium is given by $E_0 = (M^0, M_i^0, M_c^0, T^0, B^0) = (\frac{\beta}{\mu}, 0, 0, \frac{\Lambda}{\delta}, 0)$. The local stability of this fixed point is warranteed by the following result.

Theorem 4.1 *The disease-free equilibrium E_0 is locally asymptotically stable if and only if the following two conditions hold:*

i) $\epsilon\frac{\beta}{\mu} - \rho K > 0$;

ii)

$$\frac{(\epsilon\frac{\beta}{\mu})(\mu S + \eta\frac{\Lambda}{\delta}L)}{(\epsilon\frac{\beta}{\mu} - \rho K)(\alpha\frac{\Lambda}{\delta} + \eta\frac{\Lambda}{\delta} + \mu)} < 1.$$

The proof of Theorem 4.1 is given in the Appendix. Conditions (i) and (ii) are equivalent to the condition

$$\frac{\rho K}{\epsilon\frac{\beta}{\mu}} + \frac{\mu S + \eta\frac{\Lambda}{\delta}L}{\alpha\frac{\Lambda}{\delta} + \eta\frac{\Lambda}{\delta} + \mu} < 1.$$

Therefore, the basic reproductive number is

$$R_0 = R_1 + R_2,$$

where

$$R_1 = \frac{\rho K}{\epsilon\frac{\beta}{\mu}}$$

is the average number of engulfings per macrophage per lifetime of bacteria while

$$R_2 = \frac{\mu S + \eta\frac{\Lambda}{\delta}L}{(\alpha + \eta)\frac{\Lambda}{\delta} + \mu}.$$

is the average number of released bacteria per lifetime of infected macrophage.

R_1 corresponds to the engulfing, a reservoir mechanism for the bacilli. When $R_0 < 1$, the release rate of the infected macrophages is sufficiently low for the immune system to eliminate the pathogen. A strong engulfing mechanism along with a low release rate will result in an effective immune response. When $R_0 > 1$, the release rate is not sufficiently low for the immune mechanisms to eradicate the initial infection. In this case the engulfing process enables the bacteria to grow intracellularly. When this release is not sufficiently low, then some clinical manifestation of the disease may occur.

When $R_0 < 1$, (figure 1.1 and 1.2), there are three equilibrium states, two of which are endemic. A numerical bifurcation analysis (figure 2) reveals that E_1 is always locally stable. For $R_0 < 1$, the disease-free state E_0 is also locally stable, while E_3 is a saddle point. When $R_0 = 1$, then $E_0 = E_2$; the disease-free state becomes unstable as $R_0 > 1$. The model has other equilibria, but these are not biologically relevant (negative).

Parameter Values

β	=	constant recruitment rate of non-activated macrophages	1 $mm^{-3}d^{-1}$
ϵ	=	engulfing rate	4 mm^3d^{-1}
α	=	macrophage activation rate	0.008 mm^3d^{-1}
μ	=	macrophage death rate	0.003 d^{-1}
η	=	infected macrophage necrosis rate	0.008 mm^3d^{-1}
λ	=	liquefaction rate of caseous tissue	0.001 d^{-1}
Λ	=	constant T-cell recruitment rate	10 $mm^{-3}d^{-1}$
r	=	T-cell replication rate	0.02 d^{-1}
δ	=	T-cell death rate	0.007 d^{-1}
ρ	=	intrinsic growth rate of bacilli	1 d^{-1}
S	=	average number of released bacilli due to natural death of an infected macrophage	1000-2000
K	=	carrying capacity for the growth of bacteria	1000 mm^{-3}
L	=	average number of released bacilli due to the liquefaction of caseous tissue	0-1

Initial Conditions

M	=	uninfected macrophages	333 mm^{-3}
M_i	=	infected macrophages	1 mm^{-3}
M_c	=	caseous tissue	1 mm^{-3}
T	=	T-cells	1500 mm^{-3}
B	=	*M. tuberculosis*	1 mm^{-3}

Table 1: These data were taken in most part from *Dynamics of Co-infection with M. tuberculosis and HIV-1* by Denise Kirschner.

5 Discussion

5.1 Tissue-Damage and Necrosis rates

In Figure 1, lighter colors represent a greater R_0. Values for the activation rate (α) are in the x-axis and for the necrosis rate (η) in the y-axis. These numerical results arise from the parameter values in Table 4.1 and from varying the values of the activation and necrosis rates. For a disease-free state to occurr points in the darker areas should be choosen. From the graph it seems that for low necrosis rate an unfeasible macrophage activation rate may be needed. Hence the elimination of necrotic tissue may not be the answer for an effective interplay of

immunological responses. Only these two parameters were varied to look at at the possibility of an effective interplay of the two main responses were the tissue damage was minimized.

5.2 Liquefaction

The liquefaction of necrotized tissue is a process that not only releases bacteria but also enables them to grow extracellularly for the first time, permiting the spread of the disease to other main organs of the body like the liver and kidneys. We considered the proportion of released bacteria (L) in order to determine the liquefaction effect on the dynamics of the system. Interesting dynamics arise from the diferent proportions of released bacteria considered. Depending on this proportion on a given host, the disease may be perpetuated or it may result in a "quasi-equilibrium" state, that is, where the time lapse for the disease to develop was found numerically to be longer than the life span of a human being. This result, even though it was already known what the latent state is, is not only biologicaly accurate but also suggests that in order for a tuberculin-positive testing individual to not develop the disease, the ammount of liquefied tissue must be reduced toghether with the tactics used to kill bacteria inside the necrotic centers (lesions).

A Appendix

Proof of Theorem 1:

(i) From equation (5) it can be seen that

$$\rho B(K-B) - \epsilon MB \leq \frac{dB}{dt}$$

and at the initial moment of infection $\rho B \approx 0$ relative to ρK. Therefore

$$(\rho K - \epsilon\frac{\beta}{\mu})B \leq \frac{dB}{dt}$$

so that

$$B(0)e^{(\rho K - \epsilon\frac{\beta}{\mu})t} \leq B(t).$$

If $\epsilon\frac{\beta}{\mu} - \rho K \leq 0$, then $B(t)$ is bounded by below, and the bacilli will not die out.

(*ii*) The Jacobian matrix evaluated at the disease-free state is:

$$J(E_0) = \begin{pmatrix} -\mu & \alpha\frac{\Lambda}{\delta} & 0 & 0 & -\epsilon\frac{\beta}{\mu} \\ 0 & -(\alpha+\eta)\frac{\Lambda}{\delta} - \mu & 0 & 0 & \epsilon\frac{\beta}{\mu} \\ 0 & \eta\frac{\Lambda}{\delta} & -\lambda & 0 & 0 \\ 0 & \frac{\tau}{a}\frac{\Lambda}{\delta} & 0 & -\delta & 0 \\ 0 & \mu S & \lambda L & 0 & -(\epsilon\frac{\beta}{\mu} - \rho K) \end{pmatrix}.$$

Clearly $-\mu$, $-\delta$ are eigenvalues of this matrix, and they are always negative. Define the following matrix A:

$$A = \begin{pmatrix} -(\alpha+\eta)\frac{\Lambda}{\delta} - \mu & 0 & \epsilon\frac{\beta}{\mu} \\ \eta\frac{\Lambda}{\delta} & -\lambda & 0 \\ \mu S & \lambda L & -(\epsilon\frac{\beta}{\mu} - \rho K) \end{pmatrix}.$$

The following definitions are needed with the assumption $\epsilon\frac{\beta}{\mu} - \rho K > 0$ before we apply the Routh-Hurwitz criteria to A.

$$\begin{aligned} W_1 &= -Det(A), \\ W_2 &= -Tr(A), \\ W_3 &= \begin{vmatrix} -(\alpha+\eta)\frac{\Lambda}{\delta} - \mu & 0 \\ \eta\frac{\Lambda}{\delta} & -\lambda \end{vmatrix} + \begin{vmatrix} -(\alpha+\eta)\frac{\Lambda}{\delta} - \mu & \epsilon\frac{\beta}{\mu} \\ \mu S & -(\epsilon\frac{\beta}{\mu} - \rho K) \end{vmatrix} + \begin{vmatrix} -\lambda & 0 \\ \lambda L & -(\epsilon\frac{\beta}{\mu} - \rho K) \end{vmatrix}. \end{aligned}$$

W_2 is always positive while W_1 and W_3 are positive if

$$(\epsilon\frac{\beta}{\mu} - \rho K)(\alpha\frac{\Lambda}{\delta} + \eta\frac{\Lambda}{\delta} + \mu) > (\epsilon\frac{\beta}{\mu})(\mu S + \eta\frac{\Lambda}{\delta}L).$$

or equivalently iff

$$R_0 = \frac{(\epsilon\frac{\beta}{\mu})(\mu S + \eta\frac{\Lambda}{\delta}L)}{(\epsilon\frac{\beta}{\mu} - \rho K)(\alpha\frac{\Lambda}{\delta} + \eta\frac{\Lambda}{\delta} + \mu)} < 1.$$

It can be verified that whenever $R_0 < 1$ we have that $W_2 W_3 > W_1$. Therefore, by Routh-Hurwitz, the real part of all eigenvalues is negative and the disease-free state E_0 is locally asymptotically stable.

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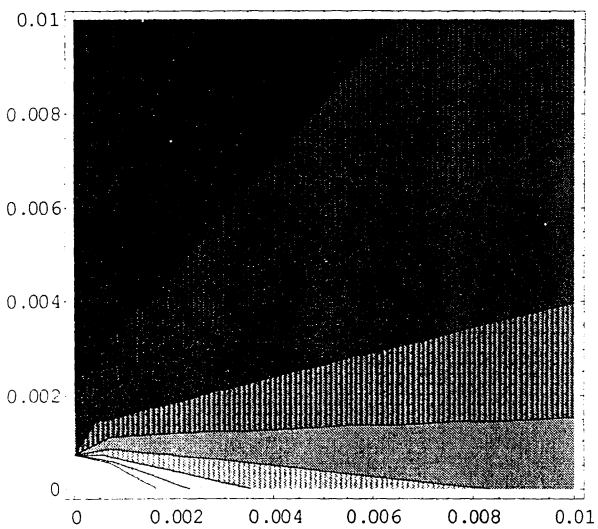

Figure 1: Tissue-Damage and Necrosis rates. Lighter colors represent a greater R_0.