Analysis of minimal embedding networks on an Immune System model

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Abstract

Immunology has always been a complex area of study with many different pathways and cellular interactions. Mathematically, this can be represented by systems of ordinary differential equations. Though analysis of ODEs is simple at first, the complexity of the interactions that take place within a large network of cells, as is the case in Immunology, makes simple analysis much more challenging. However, it has been shown that a certain immunological network not only has one equilibrium value, but under certain conditions admits multiple equilibrium values. Using a base model, I will analyze smaller, embedded subnetworks in order to find the cause of the multiple equilibrium states.

Introduction

Robust and accurate model building in biology is a very complex art. First, the biology must be understood and translated into a mathematical representation. Next, this mathematical representation has to be analyzed to determine if the model accuratly describes the biological process. Nevertheless, it is imperative that when a new model is introduced, it is analyzed by others in order to build more complex models.

In Immunology, there are many reactions that must take place in order for the Immune System to work properly. Since this area of biology is constantly changing, a base model to describe some of the simpler reactions is necessary in order to advance the field. One of the most important cells involved in

Immunology are regulatory T cells[6]. Regulatory T cells are involved in clearing most actue and chronic infections as well as indicating tolerance for the immune system[7].

Fouchet and Regeos found a crossregulation model that incorporated regulatory T cells, APCs, and effector T cells that admits multiple equilibrium states[1]. The biological meaning of bistability is tolerance. Tolerance is an unresponsive state of the immune system to a certain substance. Tolerance leads to many different biological phenomena such as allergies[1], organ transplants[4], and the acceptance of a fetus inside a mother's womb[3]. Therefore understanding the basics behind tolerance is necessary in order create more complex models describing such processes.

My work is geared towards finding the cause of the bistability from the Fouchet and Regeos regulatory T cell model. My approach is analyzing the embedded subnetworks of the original model. By doing so, I limit the scope of the biology at hand allowing me to analyze a smaller interaction network. The results gained from this type of analysis would indicate what part of the original network causes the bistability i.e. indicating some cells are more "important" than others in causing tolerance.

Background

In this section I will layout the definitions and theorem that are used throughout my research.

Creating subnetworks from the original Fouchet and Regeos model involves chemical reaction networks. Chemical reaction networks involve species, rate constants, and direction. Chemical species are the different type of reactants and products are the needed or produced. In this case, species are the different types of cells that are incorporated into the original crossregulation model. Rate constants are positive real valued numbers that describe how fast the reaction is going. Incorporating speceis and rate constants create equations that describe a certain reaction. Reactions can either be unidirectional or bidirection, so directionality is important when creating these equations. Guldberg and Waage described how to incorporate these three parameters to mathematically represent chemical reactions through what are called **mass-action kinetics** [9].

Definition 0.1. Mass-action Kinetics states that the rate of a chemical reactions is proportional to the concentration of the reacting species.

Let $c(t) = (c_1(t), ..., c_s(t))$ be concentration vectors of all the species in a chemical reaction, k_{ij} be the rate constants involved in the chemical reactions, and y_i be a vector representing the presence of each species in the reactants or products. Then the concentration vector evolves from the following differential equations:

$$\frac{dc}{dt} = \sum_{y_i \to y_j} k_{ij} c^{y_i} (y_j - y_i)$$

Example 0.1. $S_0 + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$
Using the above definition of mass-action kinetics, the corresponding differential equations for this simple reaction is:

$$\begin{aligned} \frac{dc_{S_0}}{dt} &= -k_1 c_{S_0} c_E + k_2 c_X \\ \frac{dc_E}{dt} &= -k_1 c_{S_0} c_E + k_2 c_X \\ \frac{dc_X}{dt} &= k_1 c_{S_0} c_E - k_2 c_X \end{aligned}$$

After representing a biological process as chemical reactions and transforming that information mathematically, the next step is to find an equilibrium state.

Definition 0.2. An **equilibrium state** is the state of a system when none of the species' concentration are changing. An **equilibrium state** or steady state is a unique solution of concentration vectors, $(c_1^*, c_2^*, ..., c_i^*)$ that satisfies the system of equations when all $\frac{dc_i}{dt} = 0$.

Definition 0.3. A chemical reaction network admits **multiple steady states** is there exist rate constants $k'_{ij}s \in R_{>0}$ whose resulting system admits two or more steady states i.e. two or more steady states.

This means for a system to be bistable, there must exist two unique solutions, $(c_1^*, c_2^*, ..., c_i^*)$ and $(c_1^{**}, c_2^{**}, ..., c_i^{**})$ that satifies the system when all $\frac{dc_i}{dt} = 0$.

Theorem 0.1 (Joshi and Shiu 2013)**.** *A network with inflows/outlows admits multiple seady states if and only if some embedded subnewtork admits multiple steady states.*

From this theorem, I can remove portions of Fouchet and Regeos' original model and analysis the smaller model for their steady states. If that smaller embedded subnetwork is bistable then any network that includes that smaller network will also be bistable. Therefore if one of the smaller networks that I will analyze is bistable, then I have found the cause of the bistability in the larger network. The goal is to use this theorem in order to gain further insight on the biology by analyzing the mathematics of the model.

The model Fouchet and Regeos proposed for self vs. nonself tolerance was first illustrated by Powrie and Maloy[8], represented by Figure 1

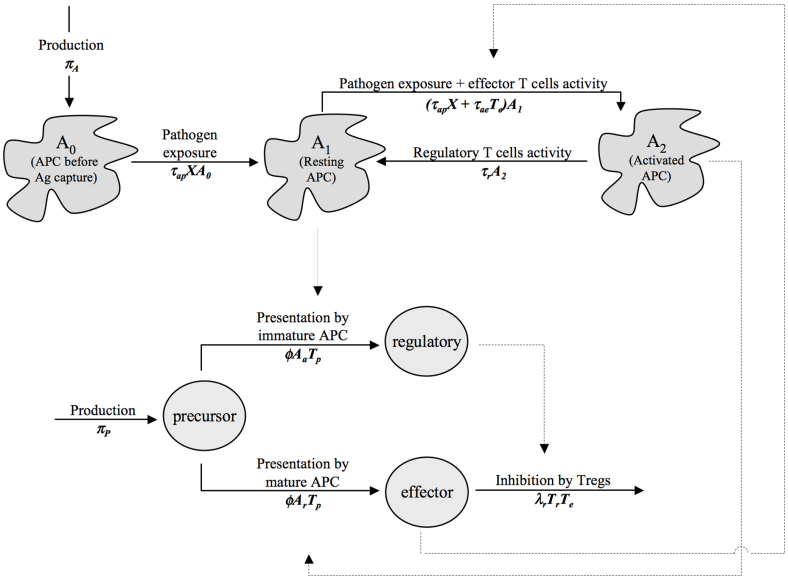


Figure 1: Immune System Network

The corresponding system of ordinary differential equations that describes this system is:

$$\frac{dX}{dt} = \pi_X - m_x X - kT_e X, \tag{1}$$

$$\frac{dA_0}{dt} = \pi_A - \tau_{ap} X A_0 - m_A A_0, \tag{2}$$

$$\frac{dA_1}{dt} = \tau_{ap} X A_0 + \tau_r T_r A_2 - [\tau_{ae} T_e + \tau_{ap} X] A_1 - m_A A_1, \tag{3}$$

$$\frac{dA_2}{dt} = -\tau_r T_r A_2 + [\tau_{ae} T_e + \tau_{ap} X] A_1 - m_A A_2, \tag{4}$$

$$\frac{dT_p}{dt} = \pi_p - m_p T_P - \phi A_2 T_p - \phi A_1 T_p, \tag{5}$$

$$\frac{dT_e}{dt} = \phi A_2 T_p - m_e T_e - \lambda_r T_r T_e, \tag{6}$$

$$\frac{dT_r}{dt} = \phi A_1 T_p - m_r T_r. \tag{7}$$

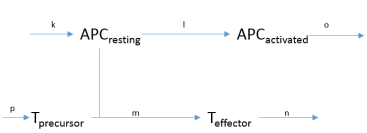
where the biological meaning of the rate constants are:

Parameter	Symbol
Inflow of antigen	π_X
Outflow of antigen	m_X
Death rate of precursor T cells	m_p
Decay rate of effector T cells	m_e
Decay rate for T_{reg} cells	m_r
Birth rate of APCs	π_A
Death rate of APCs	m_A
Killing rate for effctor T cells	k
Activation rate of APC by antigen	τ_{ap}
Reactivation rate of APC by effector T cells	τ_{ae}
Differentiation rate of precursor T cells	ϕ
Inhibition rate of effector T cells by T_{reg} cells	λ_r
Inhibition rate of APCs by T_{reg} cells	τ_r

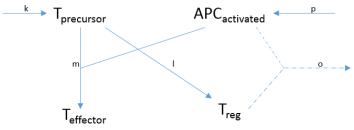
Results

How I create the subnetworks are important. I want the subnetworks to retain some biological significance therefore I defined biological significance as interactions between cell lines. This means in each subnetwork there must be interactions between APCs and some type of T cell. If there were no interactions, then the different types of cells would not evolve and no immune response could ever happen. Therefore I must restrict any subnetwork to have these types of interactions in order to preserve some sort of biological significance.

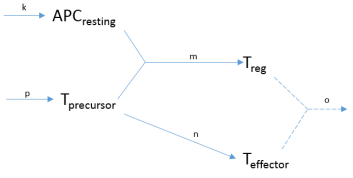
Looking at the main block of interactions, it is easy to see that four types of cells are important in this model: resting APC, activated APC, regulatory T cell, and effector T cell. Therefore I will start with four cases where I ignore one of these types of cells while considering the resulting interactions between the cell types.
These four cases can be described picturaly as:



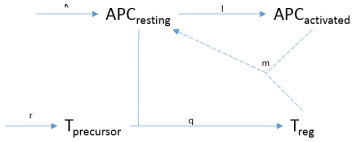
(a) Case 1: Subnetwork of all APCs and effector T cell interaction



(b) Case 2: Subnetwork of all T cell interaction with only activated APC



(c) Case 3: Subnetwork of all T cells interactions with resting APC



(d) Case 4: Subnetwork all APCs and only regulatory T cell

Figure 2: Case Study: the dashed lines represent inhibitory effects. If two lines come together then it indicates mass-action between the two reacting species. The letters above each line represent the rate constants for each chemical reaction

Next I used CoNtRoL, a web-based application which performs dynamic analytics on an inputted chemical reaction network. After inputting the previous cases into CoNtRoL, only Case 3 and Case 4 had the possibility of positive multiple equilibria. This possibility only occured when using power-law kinetics not mass-action kinetics. Power-law kinetics is different from mass-action kinetics with regard to stoichiometric equivalents of the reactants involved in the chemical reaction. In power-law kinetics there is no restriction of stoichiometric equivalence. There could be 1 stoichiometic equivalence of one reactant and 0.67 of the other reactant and the reaction could still hold. In mass-action, the stoichiometric equivalence must be 1:1 or any interger multiple of that ratio.

Nevertheless, CoNtRoL indicated that neither Case 1 nor Case 2 had the possibility of bistability, so there is no need in analyzing these cases further. The interesting outcome was that only two cases involving regulatory T cells and resting APCs interactions, Case 3 and Case 4, had the possibility of bistability.

Now I have to create a system of equations that describe both Case 3 and Case 4 and analyze them separately in order to see if these systems are indeed bistable.

I will start with Case 3. I will be changing the names of the species: $A = APC_{resting}, B = T_p, C = T_{reg}, D = T_{effector}$. The corresponding system of differential equations are as follows:

$$\dot{A} = k - mAB \tag{8a}$$

$$\dot{B} = p - nB - mAB \tag{8b}$$

$$\dot{C} = mAB - oCD \tag{8c}$$

$$\dot{D} = nB - oCD \tag{8d}$$

Since we are solving for steady states all of the differential equations are equal to 0. Now all of the species are fixed concentrations and are no longer changing, so the equilibrium state is defined by a“ *” next to the species. Therefore looking at $\dot{A}$:

$$k = mA^*B^* \Rightarrow A^* = \frac{k}{mB^*}$$

Looking at $\dot{B}$ we get:

$$p = B^*(mA^* - n) \Rightarrow B^* = \frac{p}{mA^* - n} \Rightarrow B^* = \frac{pB^*}{k - nB^*}$$

Replacing B^* with x, the polynomial equation that describes the function of B^* is $nx^2 + (p-k)x = 0$. This is a simple quadratic that always crosses the x-axis at x=0 and some other point based on the values of n, p, and k. But for the equilibrium state to be biologically relevant, x must be a positive real number when it crosses the x axis. There are only two cases when this can occur. If $n > 0$ then $p < k$, and if $n < 0$ then $k < p$. However n can't be negative, because a rate constant is never a negative real value. Either way, B^* can only admit one positive real solution.

Looking at $\dot{D}$ we have:

$$nB^* = oC^*D^* \Rightarrow D^* = \frac{nB^*}{oC^*}$$

B^* can only be a unique positive solution so D^* is a function of C^* and vice-versa. There is no way to pin point which value D^* will be theoretically. Therefore I used Matlab's fsolve function to solve the system of equations described in equations (8a)-(8d) under the condition $n > 0$, and $p < k$. I used many different intial conditions but the resulting equilibrium state was always the same. The only unique equilbrium state was located at $(A^*, B^*, C^*, D^*) = (1.59, 1.25, 0.5, 0.9)$. The result of my analysis for this subnetwork is that there is no possibility of bistability using mass-action kinetics.

With Case 4, I changed the names of the species: $A = APC_{resting}, B = T_p, C = T_{reg}, D = T_{effector}$. The corresponding system of differential equations are:

$$\dot{A} = k + mBD - lA - qAC \tag{9a}$$

$$\dot{B} = lA - mBD \tag{9b}$$

$$\dot{C} = r - qAC \tag{9c}$$

$$\dot{D} = qAC - mBD \tag{9d}$$

Just like the analysis in Case 3, solving for the equilibrium states involves setting all the differential equations equal to zero and trying to find explicit values for each species.

$$\dot{C} + \dot{D} : r = mB^*D^* \Rightarrow B^* = \frac{r}{mD^*},$$
$$\dot{B} : lA^* - r = 0 \Rightarrow A^* = \frac{r}{l},$$
$$\dot{C} : r = qAC^* \Rightarrow r = q\frac{r}{l}C^* \Rightarrow C^* = \frac{l}{q},$$
$$\dot{A} : k - lA - \dot{D} \Rightarrow k - lA^* = 0 \Rightarrow A^* = \frac{k}{l}$$

Therefore, for the system to have any equilibrium state, the two rate constants k and r must be equal to each other. Since there is a conservation relationship in this system we can analyze B^* and D^*. A conservation relationship is when the change in concentration of multiple species add up to a constant. This means that the concentration of certain species remain constant throughout the time of the reaction. The conservation realtionship in this system is $\dot{A} + \dot{D} - \dot{B}$.

From $\dot{D}$, we know $qAC = mBD$, and from $\dot{C}$ we know the $r = qAC$. Therefore $r = mBD$ and we can rewrite $\dot{B} = lA - r$. Remembering that $k = r$ is the condition that must hold in order to have any equilibrium states, $\dot{B} = lA - k$. Thus it is easy to see that

$$\dot{A} + \dot{D} - \dot{B} = 0$$

Since this conservation relationship is true at all times, $A + D - B =$ constant.

However at equilibrium this equation turns into:

$$\overbrace{A^*}^{\text{constant}} + D^* - B^* = 0$$

A^* is a constant, equal to $\frac{k}{l}$, therefore $B^* - D^* =$ constant. We know that B^* is inversely related to D^* for all possible values of D^*, and vice-versa. However if we fix the constant that $B^* - D^*$ is equal to, then we can explicitly find the equilbirum values for B^* and D^*.

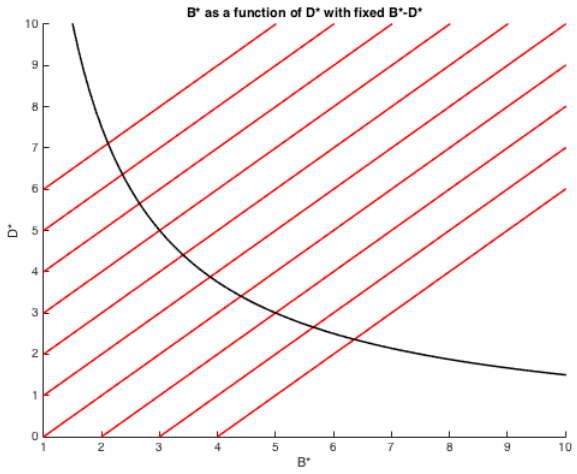


Figure 3: B^* as a function of D^* (black graph) with A^*,C^* fixed and different constant values where chosen for $B^* - D^* =$ constant (red graphs)

As shown in Figure 3, no matter what constant values are chosen for what $B^* - D^*$ is equal to, there can only be one unique equilibrium state for both B^* and D^*. Since A^* and C^* are explict solutions based on rate constants and the concentration of what B and D cannot equal two different values at one time, there can only be one unique equilbrium state for this subnetwork.

Therefore I have shown that in all four cases of subnetworks that retain biological meaning, the two that had the possibility of bistability actually can not admit bistability.

Discussion

Recall Theorem 0.1, it requires that any embedded subnetwork include all inflows and outflows in order for the theorem to apply. I however, did not include outflows in any of my subnetworks. The reason behind this is because when I included outflows from every species in my subnetworks, the possibility of bistability was lost in Case 3 and Case 4, based on CoNtRoL's analysis. Therefore if I included outflows in any of my models, then I would have no intital guess on which subnetwork to start my analysis. This is in accordance to recent findings on the subject, because the addition of arbitrarily

small inflows and outflows has been shown to lose bistability in a chemical reaction network[2]. Since others have proved that adding in small amounts of outflows would cause the network to lose its ability to be bistable, I ignored outflows until I could find the cause of the bistability. Then once that was found, I would add them back in in order to analyze the subnetworks corectly. Interestingly enough, this was not necessary, because none of my subnetworks admitted bistability without outflows.

This result disproves my intuition and motivation of my research. I wanted to find a reason why the original Fouchet and Regeos model was bistable and in doing so I would be able to gain further insight on the role of regulatory T cells interactions. However, my research has shown that using the subnetwork approach to find the cause of bistability only disproves bistability in each subnetwork. Although this is not what I had indended to find, results are nevertheless results whether "good" or "bad". Though my research was counterintuitive in nature (usually these types of equilibrium analysis are straighforward with the result being an explicit solution to what is trying to be proved), it has a positive repercussion. In a sense, my analysis validates the original model from Fouchet and Regeos as being the most concise and precise system of equation that describes the role of regulatory T cells in self vs. nonself tolerance. Since each subnetwork could not admit bistability, every interaction and cell type from the original model is necessary in order for bistability to occur. This means that any future model used to describe tolerance should include all of the cells, and interactions that Fouchet and Regeos outlined in their paper. When a new mathematical model is introduced, the biology is deemed so grand that extraneous species and cell interactions are usually added into the model. Although my intial intention was not to prove the Fouchet and Regeos model, it turned out that my research does indeed validate the correctness and succinctness of their model.

As a side note, I thought that the naive APC cell type was unecessary when I first looked at the Fouchet and Regeos model. I could not mathematical take out the naive APC from the original system of equations and have the resulting system describe the same biology. I thought of the inflow into the naive APC and the outflow of the naive APC into the resting APC as one big inflow. This technique could not be substituted into the system of equation, because of the mass action term of the pathogen exposure and naive APC. Notice that all of my subnetworks do not include naive APC. I did initially

have subnetworks with them involved, but the results from CoNtRoL were the same. Only Case 3 and Case 4 with naive APC could have the possibility of bistability, thus in order to find the smallest or minimal embedding network, I left out the naive APC from my subnetworks. I leave it to the reader to find a way to reduce the original Fouchet and Regeos model by taking out the naive APCs, because it does not seem to have any effect of the bistability or the presence of equilibrium values.

Ackonwlegements

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