

Functional Switching and Stability of Regulatory T Cells

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Abstract It is widely accepted that the primary immune system contains a subpopulation of cells, known as regulatory T cells whose function is to regulate the immune response. There is conflicting biological evidence regarding the ability of regulatory cells to lose their regulatory capabilities and turn into immune promoting cells. In this paper, we develop mathematical models to investigate the effects of regulatory T cell switching on the immune response. Depending on environmental conditions, regulatory T cells may transition, becoming effector T cells that are immunostimulatory rather than immunoregulatory. We consider this mechanism both in the context of a simple, ordinary differential equation (ODE) model and in the context of a more biologically detailed, delay differential equation (DDE) model of the primary immune response. It is shown that models that incorporate such a mechanism express the usual characteristics of an immune response (expansion, contraction, and memory phases), while being more robust with respect to T cell precursor frequencies. We characterize the affects of regulatory T cell switching on the peak magnitude of the immune response and identify a biologically testable range for the switching parameter. We conclude that regulatory T cell switching may play a key role in controlling immune contraction.

Keywords Immune regulation · Functional switching

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1 Introduction

The magnitude and effectiveness of an immune response depends largely on the balance of positive and negative signals communicated by various components of the immune system. While the initial phases of T cell expansion are well understood (see Murphy et al. 2008), the final contraction phase of the immune response is not. This contraction phase is crucial in bringing the immune system back to a stable state. Such a contraction must happen quickly to prevent damage to healthy cells and to prepare the system for future challenges. Hence, cell-mediated immune responses must be closely regulated following any antigenic stimulation.

Regulatory T cells (Tregs) play a key role in the contraction of the immune response. These cells are recruited and activated during an adaptive immune response and are critical in preventing excessive immune reactions (Sakaguchi 2010). Along with the role they play in contracting a normal immune response, an additional function of regulatory T cells (Tregs) is to regulate immune responses that may lead to autoimmunity. Regulatory T cells comprise approximately 5–10 % of the mature CD4+ T cell subpopulation in mice and humans. They differentiate from varying sources and are affected by a number of immunosuppressive cytokines (such as TGF- β and IL-10) as well as immunostimulatory cytokines (e.g., IL-2) (Sakaguchi 2010; Murphy et al. 2008).

In a recent *Nature* article, Sakaguchi outlines recent biological evidence regarding the stability of regulatory T cells (Tregs) (Sakaguchi 2010). In the past, the T cell lineage has been assumed to progress downstream towards more differentiated cells. Contrary to this dogma, Tregs were recently discovered to be able to differentiate “sideways,” halting their regulatory function and becoming effector T cells that secrete pro-inflammatory cytokines. Immunologists are currently working toward characterizing the conditions under which this conversion may happen. In order to understand how regulatory T cells regulate immune responses, it is imperative to investigate how they are produced and the conditions under which they are functionally stable.

While many mathematical models examine T cell lineage and differentiation (Callard et al. 1999; Fishman and Perelson 1999; Hong et al. 2011; Yates et al. 2000), here we develop a mathematical model that includes the novel consideration that regulatory T cells may lose their regulatory capabilities. Previous work has used a number of mathematical techniques in the areas of nonlinear dynamical systems and agent based modeling to investigate questions such as the Th1 versus Th2 immune responses (Fishman and Perelson 1999; Yates et al. 2000) and immune cross-regulation (Callard et al. 1999; Hong et al. 2011). In this work, we are investigating how immune regulation arising from regulatory T cells is affected by a feedback mechanism in which helper T cells may become regulatory T cells and vice versa.

A number of models have been developed to describe the cell-mediate immune response. Initial models of the dynamics of the immune response were based on predator-prey type dynamics wherein immune cells acted as predators and pathogens served the roll of the prey (e.g. Althaus et al. 2007). These models are able to generate some of the general features of the immune response such as expansion/contraction dynamics. However, they fail to exhibit some key experimentally-obtained features of the immune response. For example, experimental evidence suggests that the T cell response is mostly dictated in the first hours of antigen exposure and is therefore largely

insensitive to the later characteristics of antigen exposure (Kaech and Ahmed 2001; Mercado et al. 2000; van Stipdonk et al. 2003). Mathematically, this has led to approaches rooted in a concept of T cell expansion wherein T cells enter a predetermined program where they divide for either a specified period of time or specific number of divisions (Antia 2003; Kim et al. 2010; Wodarz and Thomsen 2005). In Kim et al. (2010), Kim et al. demonstrated that any preprogrammed response must be proportional to the precursor frequencies. Such scaling contradicts the experimental evidence of Badovinac et al. (2007). They then proposed a shift in paradigm wherein the contraction of effector T cells is the result of negative feedback from adaptive regulatory T cells. These regulatory T cells develop in the course of an immune response and create a negative feedback loop that suppresses effector cells. Mathematical models developed under this hypothesis are given in Kim et al. (2010, 2011, 2012).

In this paper, we develop two mathematical models of the primary immune response with the goal of characterizing the effects of regulatory T cell switching on the immune dynamics. The paper is organized as follows: In Sect. 2.1, we review the various hypotheses concerning immune contraction and argue in favor of active suppression of immune responses by regulatory T cells. Section 2.2 describes the biological system highlighted by Sakaguchi and presents the experimental evidence used as a basis for our Treg switching mechanism. In Sect. 3.1, we study regulatory T cell switching in the context of a simplified ODE model that considers only helper and regulatory T cell dynamics. A further exploration of this mechanism in the context of a more biologically detailed model of the immune response is carried out in Sect. 3.2. Model simulations and numerical experiments characterizing the effects of Treg switching are given in Sect. 4. Closing remarks and directions for future work are given in Sect. 5.

2 Biological Background

2.1 Immune Regulation via Adaptive Regulation

The mechanisms by which the immune system contracts after an infection are widely debated. Razvi et al. demonstrated that apoptosis is the principal mediator of T cell contraction (Razvi et al. 1995). Their experiments showed that the highest rate of apoptosis occurred at the peak of the T cell response. This led them to conclude that cells are programmed to proliferate for a fixed amount of time, after which they die (Razvi et al. 1995). An alternative point of view focuses on a division-based cell program. In this approach, cells are thought to be programmed to divide a given number of times before undergoing apoptosis (see, e.g., Renno et al. 1999). An alternative theory is that of adaptive regulation: During the course of an immune response, negative feedback from regulatory T cells suppresses the activation of conventional T cells thereby retracting the immune response (Taams et al. 2002).

The theory of adaptive regulation is supported by the work of Taams et al. who concluded that active suppression of T cells by regulatory T cells is plausible due to evidence that passive competition of resources (access to APCs) cannot account for the decay of the immune response (Taams et al. 2002). It has been known for many

years that regulatory T cells exist in the lymph nodes, but in 2004, Baecher-Allan et al. demonstrated that Tregs are also present in the periphery of humans (Baecher-Allan et al. 2004). These peripheral Tregs can come from naïve T cells that have acquired a Foxp3 expression (Apostolou and vonBoehmer 2004; Chen et al. 2003; Kretschmer et al. 2005) or by trafficking from the lymph nodes to the site of inflammation using homing receptors similar to those used by other effector T cells (Belkaid et al. 2002; Huehn 2005; Sather et al. 2007). It has also been shown that natural Tregs can expand clonally following antigenic stimulation and retain their suppressive function after expansion (Fehérvári and Sakaguchi 2004; Klein et al. 2003; Yamaguchi et al. 2007; Yamazaki et al. 2003). Regulatory T cells have also been shown to suppress the proliferation and differentiation of naïve T cells. They can suppress the activities of mature helper T cells, mature cytotoxic T cells, natural killer cells, B cells, macrophages, and dendritic cells (Miyara 2007; Shevach 2006; Tang et al. 2006; von Boehmer 2005). The experimental evidence supports the notion that regulatory T cells exist at the site of an infection either through the homing of naturally occurring Tregs or through the induction of naïve T cells in the periphery.

Just like other T cells, Tregs activate and proliferate upon antigen stimulation. After expansion, they retain the ability to suppress many of the immune cells involved in the immune response.

2.2 Concerning Treg Stability

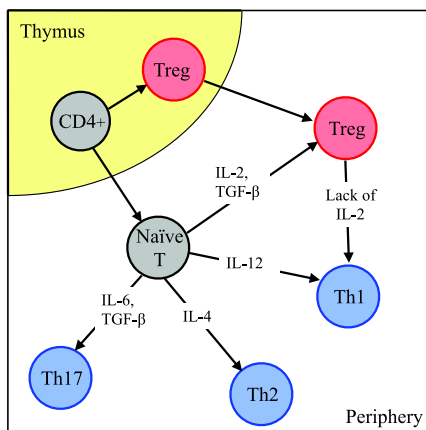
A number of cytokines are essential to regulatory T cell function. These include the immunosuppressive cytokines TGF- β , Interleukin-10 (IL-10), and the immunostimulatory cytokine Interleukin-2 (IL-2). IL-2, the majority of which is derived from Th1 effector T cells, is indispensable for the maintenance of Foxp3+ Treg cells and is functionally essential for Treg development. IL-2 maintains Foxp3+ natural Tregs, triggers cell expansion at high doses, and facilitates TGF- β dependent differentiation of naïve T cells to Foxp3+ Tregs. Collectively, these factors ensure a remarkably constant number of Foxp3+ Treg cells in the immune system (about 10 % of all T cells expressing the surface marker CD4), with a general increase only at sites of inflammation (Sakaguchi et al. 2008; Sakaguchi 2010).

In Sakaguchi (2010), Sakaguchi outlines experimental findings concerning the interactions between cytokine signaling, helper cell differentiation and the conditions under which regulatory T cells might convert into pro-inflammatory cells. A diagram of CD4+ T cell differentiation along with the cytokines that mediate that of differentiation is shown in Fig. 1. Interleukins 2 and 12 drive Th1 cell production. Th1 cells are specialized at confronting intracellular pathogens. Interleukins 4 and 10 inhibit Th1 cell production while promoting the Th2 phenotype, which works toward the elimination of extracellular pathogens. Th17 arise from a combination of Interleukin-6 and TGF- β signaling. These cells produce IL-17 and contribute to microbial immunity.

Treg conversion, the process of regulatory cells halting expression of Foxp3 and secreting pro-inflammatory cytokines, may happen only under certain conditions. The experimental evidence highlighted in Sakaguchi (2010) is as follows:

1. Regulatory T cells vary in Foxp3 expression and, therefore, in their susceptibility to conversion. While it is possible that all regulatory T cells are subject to conversion, it is also plausible that only a certain fraction of Foxp3+ Tregs may be

Fig. 1 CD4+ T-cell differentiation. The thymus produces naïve T cells, which can differentiate into effector T cells, including Th1, Th2, Th17 cells, and Tregs following stimulation with antigens. In the periphery, the balances of TGF- β and IL-2 determine whether a helper T cell becomes a regulatory T cell and whether a regulatory T cell becomes a helper T cell



- plastic (Zhou et al. 2009). This could be due to variations in Foxp3 expression due to T cell lineage, maturity, genetic and environmental factors (Huehn et al. 2009; Zhou et al. 2009).
2. Rubtsov et al. show that Treg cells are highly stable under certain conditions in terms of both Foxp3 expression and their suppressive function, with few cells converting into effector T cells (Rubtsov et al. 2010). Sakaguchi points out that this is not necessarily contradictory to previous known data and outlines ways in which this data can be consistent with other experiments.
 3. When Foxp3+ Treg cells are transferred to T-cell-deficient mice, the cotransfer of T cells that do not express Foxp3 or the infusion of IL-2 prevents conversion of the Foxp3+ Treg cells to effector T cells. If Treg cells are transferred on their own to T cell deficient mice, Foxp3- T cells that have formed from Foxp3+ Treg cells will produce IL-2, which inhibits further conversion of Foxp3+ Treg cells in a negative-feedback loop (Duarte et al. 2009).

3 Mathematical Models of Adaptive Regulation and Treg Switching

3.1 An ODE Model of Functional Treg Switching

We begin our mathematical study of functional Treg switching by considering a simplified version of the biological system described in Sect. 2.2. A schematic diagram of the reduced biological system is shown in Fig. 2. In this model, we consider two cell populations: helper T cells and regulatory T cells. The helper T cell compartment combines the three types of helper T cells: Th1, Th2, and Th17 while the regulatory T cells compartment encompasses both naturally occurring and antigen induced regulatory T cells. We consider two cytokines that are primarily responsible for the facilitation of the transition between these cells. These are the pro-inflammatory cytokine, IL-2 and the pro-regulatory cytokine TGF- β .

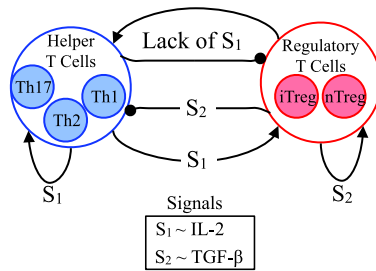


Fig. 2 A simplified version of the biological system represented in Fig. 1. This is the basis for our mathematical model. There are two cell populations: helper T cells and regulatory T cells. The helper T cell compartment combines the three types of helper T cells: Th1, Th2, and Th17 while the regulatory T cells compartment encompasses both naturally occurring and antigen induced regulatory T cells. Two main signaling proteins mediate this transition. They are IL-2, represented by S_1 and TGF- β , represented by S_2

A mathematical model that corresponds to Fig. 2 can be written as the following system of ODEs:

$$\frac{dH}{dt} = a(t) + \gamma \frac{S_1 H(1 - k_1 H)}{1 + cS_2} + \mu \frac{R}{1 + dS_1} - \delta_0 H - \delta_1 RH, \tag{1}$$

$$\frac{dR}{dt} = a(t) + \nu S_1 S_2 R(1 - k_2 R) - \mu \frac{R}{1 + dS_1} - \delta_0 R, \tag{2}$$

where

$$S_1 = \xi H \quad \text{and} \quad S_2 = \beta R.$$

In this model, we follow the dynamics of helper T cells, denoted $H(t)$; and regulatory T cells, denoted $R(t)$. We model the signals between these cells in a way similar to Yates et al. (2000). We assume that the signals S_1 and S_2 are being produced by helper and regulatory T cells, respectively. T cell recruitment and activation depend on these cytokines. S_1 represents those cytokines predominately produced by helper T cells. Therefore, although S_1 should be thought of as primarily representing IL-2 levels, it could also represent a myriad of proteins including IL-4, and IFN- γ . Similarly, S_2 represents the proteins that are predominantly produced by regulatory T cells. These include TGF- β and IL-10. We consider the magnitude of these signals to be proportional to the number of cells that produce them, and hence, $S_1 = \xi H$ and $S_2 = \beta R$. The function $a(t)$ is used to simulate recruitment of cells as a result of antigen presentation. This function is a Gaussian-like function, starting from 0, stays positive for some time, and returns to 0.

The dynamics of helper T cells is described by Eq. (1). The second term on the RHS of (1), $\gamma S_1 H(1 - H)(1 + cS_2)^{-1}$, describes the immune recruitment and proliferation of helper T cells. These cells are modeled as proliferating following a logistic growth rate. This growth is then modified by the present signals. S_1 signals are autostimulatory for helper T cells, while S_2 signals are regulatory. The factor $\gamma S_1(1 + cS_2)^{-1}$ multiplying the logistic growth of helper T cells reflects the effects these signals have on helper T cell recruitment and proliferation. The multiplicative factor S_1 accounts for the autostimulatory effects of this population and $(1 + cS_2)^{-1}$

accounts for the negative effects of the regulatory signals. The second term describes the creation of helper T cells that have originated from regulatory T cells that have lost their regulatory capabilities. Pro-inflammatory S_1 signals are required for the maintenance of regulatory T cell function. Hence, this switch from a regulatory T cell to a helper T cell occurs when there is a lack of pro-inflammatory signals produced by helper T cells. The final two terms of this equation model the removal of helper T cells from the system. These cells have both a natural death rate; assumed to be the natural death rate for all effector cells, δ_0 ; and an adaptive death/removal rate that is proportional to the mass action interaction with regulatory T cells, δ_1 .

Equation (2) describes the concentration of Tregs in the regulatory T cell compartment. Regulatory proliferation and recruitment depends both on autostimulatory signals from other regulatory T cells and on signals coming from immune promoting cells. These interactions are described by the second term of Eq. (2). Regulatory T cells are modeled as proliferating at a logistic growth rate with multiplicative factors, S_1 and S_2 indicating the presence of the co-stimulatory signals needed to facilitate this proliferation. The term $-\mu R(1 + dS_1)^{-1}$ is the negative of the corresponding Treg switching term in Eq. (1). Lower levels of S_1 signals creates higher rates of transition from regulatory to helper T cells while higher prevalence of S_1 helps to sustain regulatory cells and decreases the rate of transition. Regulatory T cells have a natural death rate δ_0 .

3.2 A Model of the Primary Immune Response with Adaptive Regulation and Treg Switching

We extend our study on the primary immune response by including both active T cell suppression and Treg switching in a more biologically detailed model of the regulation of the primary immune response. Kim et al. (2010, 2011) present a mathematical model of the immune response including adaptive regulatory T cells and show these cells they may play a crucial role in inducing a timely and robust contraction of the T cell response. In Kim et al. (2010), the regulation of cytotoxic T cells was considered. The T cell population was not divided into helper T cells and CTLs. The model was extended in Kim et al. (2011, 2012) to consider the regulation of helper and cytotoxic cells separately. This model is consistent with the theory of active suppression, displays many inherent immune characteristics (antigen and T cell precursor-independence), and thus provides an excellent framework within which to consider Treg switching. We use the work of Kim et al. (2012) as the basic model to which we add terms that allow for regulatory T cell switching.

The model developed in this paper is based on the following assumptions:

1. The T cell response is mostly determined in the first hours of antigen presentation (Kaech and Ahmed 2001; Mercado et al. 2000; van Stipdonk et al. 2003).
2. The T cell response is insensitive to precursor T cell frequencies (Badovinac et al. 2007).
3. Regulatory T cells are the mechanism of contraction following pathogen clearance (see Sect. 2.1).
4. Regulatory T cells have the ability to change their functionality and become immune promoting helper T cells (Sakaguchi 2010).

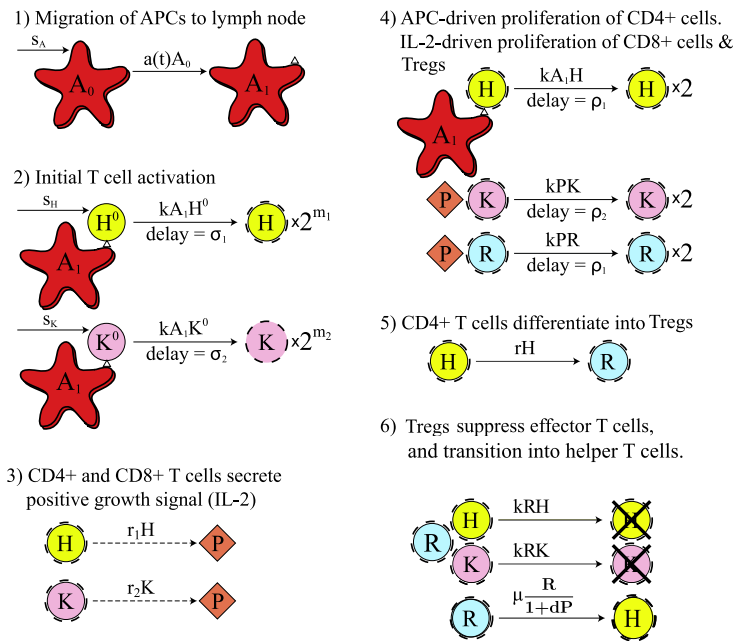


Fig. 3 Adaptive regulation model with T cell switching (3)–(10). The compartments are represented as follows: A_0 : immature APCs, A_1 : mature APCs, H^0 : naïve helper T cells, H : mature helper T cells, K^0 : naïve effector T cells, K : mature cytotoxic T cells, P : IL-2 cytokine, and R : regulatory T cells. Each compartment has an associated death rate, which is not represented in the diagram

The immune reaction is modeled as a system of DDEs. The delay is derived from the notion that upon activation, a cell will spend a specified amount of time solely devoted to mitosis (cell division) before it can properly execute its functions. This model also considers the production and consumption of the pro-inflammatory cytokine IL-2. Regulatory T cells differentiate from helper T cells and are able to suppress both helper and cytotoxic T cells (Sakaguchi 2010). Our model is described as follows (see Fig. 3):

1. Upon encountering antigen, $a(t)$, immature APCs, $A_0(t)$, become mature APCs, $A_1(t)$, and migrate to the lymph node.
2. Naïve helper and cytotoxic T cells ($H^0(t)$ and $K^0(t)$, respectively) residing in the lymph nodes encounter mature APCs and enter a minimal developmental program in which they divide m_1 or m_2 times, respectively.
3. Mature helper and cytotoxic T cells ($H(t)$ and $K(t)$, respectively) both secrete the positive growth signal IL-2, denoted $P(t)$.
4. Mature helper and cytotoxic T cells that have completed the minimal developmental program become effector cells that continue dividing upon further antigenic stimulation. This antigen dependent proliferation occurs in response to interactions with APCs for helper T cells and in response to IL-2 consumption for cytotoxic T cells.

5. In response to antigenic stimulation, some proportion of helper T cells further differentiate into Tregs, $R(t)$.
6. Regulatory T cells
 - (a) suppress mature helper and cytotoxic T cells,
 - (b) proliferate after consuming free positive growth signal,
 - (c) transition back into the helper T cells at a rate that depends on the growth signals received from mature T cells.

A model of adaptive regulation with regulatory T cell switching is thus given by the following equations:

$$\dot{A}_0(t) = s_A - d_0 A_0 - a(t) A_0(t), \tag{3}$$

$$\dot{A}_1(t) = a(t) A_0(t) - d_1 A_1(t), \tag{4}$$

$$\dot{H}^0(t) = s_H - \delta_0 H^0(t) - k A_1(t) H^0(t), \tag{5}$$

$$\begin{aligned} \dot{H}(t) = & 2^{m_1} k A_1(t - \sigma_1) H^0(t - \sigma_1) - k A_1(t) H(t) + 2k A_1(t - \rho_1) H(t - \rho_1) \\ & + \mu \frac{R(t)}{1 + dP(t)} - (\delta_H + r) H(t) - kR(t) H(t), \end{aligned} \tag{6}$$

$$\dot{K}^0(t) = s_K - \delta_0 K^0(t) - k A_1(t) K^0(t), \tag{7}$$

$$\begin{aligned} \dot{K}(t) = & 2^{m_2} k A_1(t - \sigma_2) K^0(t - \sigma_2) - k_E P(t) K(t) + 2k P(t - \rho_2) K(t - \rho_2) \\ & - \delta_K K(t) - kR(t) K(t), \end{aligned} \tag{8}$$

$$\dot{P}(t) = r_1 H(t) + r_2 K(t) - \delta_P P(t) - kP(t) K(t) - kP(t) R(t), \tag{9}$$

$$\begin{aligned} \dot{R}(t) = & r H(t) + 2k P(t - \rho_1) R(t - \rho_1) - kP(t) R(t) \\ & - \mu \frac{R(t)}{1 + dP(t)} - \delta_H R(t). \end{aligned} \tag{10}$$

A_0 is the concentration of APCs at the site of infection and A_1 is the concentration of APCs that have matured and begun presenting antigen in the lymph node. The variable H_0 is the concentration of naïve helper T cells. H is the concentration of mature helper T cells. K_0 is the concentration of naïve cytotoxic T cells, K is the concentration of mature cytotoxic cells, and R is the concentration of Tregs. The growth signal, P , is the concentration of interleukin-2.

Equation (3) describes the immature APCs maintained throughout the body. There is a constant supply rate, s_A and proportional death rate, d_0 . The invasion by an immunogen is represented by the rate, $a(t)$ defined by (11), with which the immunogen stimulates immature APCs to become mature APCs. The equation governing mature APCs in the lymph node is given by (4). The source of these cells is immature APCs that have been stimulated by antigen contact. These cells have a natural death rate of d_1 .

Naïve T cell populations are described by Eqs. (5) and (7). Helper and cytotoxic T cells have a supply rate of s_H and s_K , respectively, and die at a proportional rate δ_0 . Each of these populations mature at a rate proportional to their mass action interactions with mature APCs.

Equation (6) characterizes the mature helper T cell population. The first term gives the rate at which activated naïve helper T cells become mature upon completion of the minimal developmental program of m_1 cell divisions. The duration of this minimal developmental program is signified by the time delay σ_1 . The second and third terms correspond to antigen dependent proliferation and represents the rate at which these cells are stimulated by mature APCs for further division. The time delay of ρ_1 is the duration of one CD4+ cell division. The fourth term describes the influx of helper T cells that have originated from regulatory T cells that have lost their suppressive capabilities. This term is one of the additions to the model of Kim et al. (2012) due to the assumed Treg switching. Mature helper cells are removed from the system by three methods: natural death at rate δ_H , differentiation into Tregs at rate r , or by suppression due to interactions with Tregs.

Equation (8) governs mature CTLs. The first term of this equation gives the rate at which activated naïve CD8+ T cells enter the mature population after finishing the minimal developmental program of m_2 cell divisions. The duration of the minimal developmental program is given by the time delay σ_2 . The second term is the rate at which these cells are stimulated by IL-2 for further division, and the third term is the rate at which cells reenter the mature CTL population after dividing once in the time period ρ_2 . CTLs die naturally at a rate δ_K and are suppressed by Tregs.

The dynamics of IL-2 concentration are given by Eq. (9). This cytokine is produced by mature helper and cytotoxic T cells at rates r_1 and r_2 , respectively. The cytokine has a proportional decay rate, δ_P . The final terms of this equation describe the rates at which IL-2 is consumed by mature CTLs and Tregs.

The final equation, (10), describes the regulatory T cell compartment. The first term is the rate at which mature helper T cells differentiate into Tregs. The second and third terms describe the rate at which Tregs are stimulated by IL-2 for further division. Upon stimulation, Tregs are removed from the system at time, t , divide and return with a delay of ρ_1 . The fourth term describes the rate of loss of regulatory function. As described in Sect. 3.1, this rate is dependent on the availability of IL-2. Lower values of $P(t)$ create higher rates of transition from regulatory to helper T cells while higher prevalence of $P(t)$ helps to sustain regulatory cells and decreases the rate of transition. Tregs also have a natural death rate δ_H .

4 Results

All simulations of the ODE systems were carried out using ODE45 in Matlab. For the system of DDEs, we used the Matlab solver DDE23.

4.1 Functional Treg Switching: Numerical Results and Simulations

We begin by demonstrating that the model (1)–(2) can capture some of the key characteristics of the immune response. After validating the model, we proceed by considering the implications of Treg functional switching within the model.

Fig. 4 Antigen stimulation function, $a(t)$, for $b = 5, c = 4$, and $b = 10, c = 3$

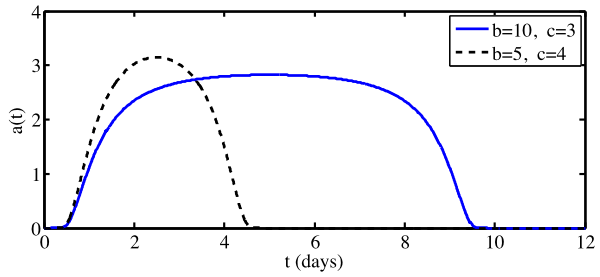
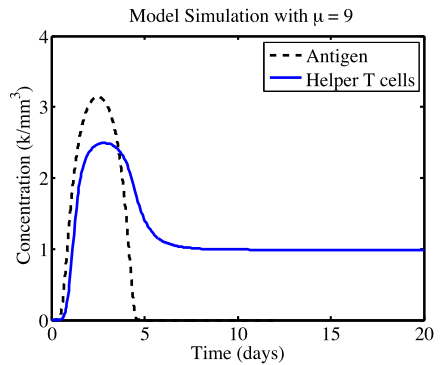


Fig. 5 Simulation of (1)–(2) with parameters from Table 1. The dynamics of the helper T cells are shown in the *solid line*. Antigen presentation, $a(t)$, with $b = 4, c = 5$ is shown with a *dashed line*



For the function representing antigen presentation, $a(t)$, we follow (Kim et al. 2010) and define

$$\phi(x) = \begin{cases} e^{-1/x^2}: & x \geq 0, \\ 0: & x < 0, \end{cases}$$

and set

$$a(t) = \frac{c\phi(t)\phi(b - t)}{\phi(b)^2}, \tag{11}$$

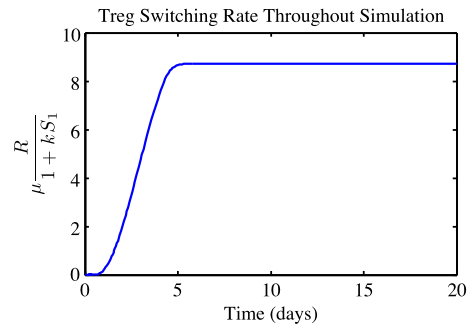
where $b, c > 0$. Graphs of $a(t)$ for $b = 5, c = 4$, and $b = 10, c = 3$ are shown in Fig. 4.

Figure 5 shows a simulation of (1)–(2) with $b = 5, c = 4$. The parameters used in this simulation are shown in Table 1. These parameters are chosen to demonstrate the characteristics of the model. They are not biologically motivated. A choice of parameters that is directly tied with the biology is conducted for the more comprehensive model (3)–(10). The dynamics of the simulated system seem to be similar over across many values of b and c . In accordance with the antigen stimulation, we identify a growth of the helper T cells peaking soon after the peak of antigen presentation. After antigen clearance, there is a contraction of immune cells leading to a positive steady state. These lingering helper T cells correspond to the emergence of memory T cells. As a model of the cellular immune process, these results agree with the expansion, contraction, and memory dynamics seen in other mathematical models of the primary immune response (Antia 2003; Kim et al. 2010;

Table 1 Parameter values used in simulations of (1)–(2). These parameters are not based on any direct biological data. Concentrations are measured in k/mm^3 . Time is measured in days

Parameter	Description	Estimate
γ	Immune recruitment rate of helper T cells	1
c	Inhibitory rate of TGF- β on helper T cell recruitment	1
μ	Magnitude of regulatory T cell switching rate	9
d	Magnitude of dependence of Treg switching on helper T cell signals	1
δ_0	Natural death rate of effector T cells	0.01
δ_1	Death rate of helper T cells as a result of interaction with Tregs	0.01
ν	Immune recruitment rate of regulatory T cells	1
ξ	Multiplicative factor of helper T cell signals	1
β	Multiplicative factor of regulatory T cell signals	0.5

Fig. 6 A graph of the magnitude of the transition rate of regulatory T cells due to helper T cell signals for the simulation shown in Fig. 5

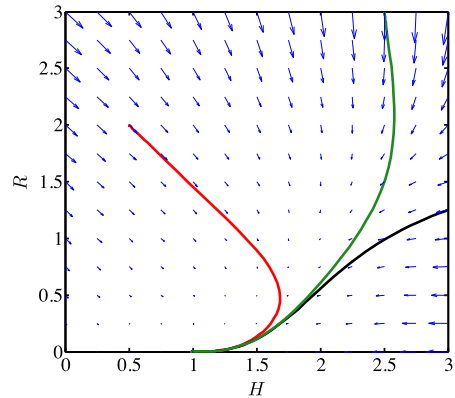


Nowak 1996). We conclude that model, with the consideration of the new regulatory switching mechanism, displays the expected expansion/contraction dynamics.

We now study the effects of the regulatory T cell switching. What is the contribution of the $\mu R(1 + kS_1)^{-1}$ term to the system? Figure 6 shows how the magnitude of the Treg switching term changes over the course of simulation. The death rate of Tregs due to lack of signaling gradually increases as the immune response progresses. While the antigen is present, and helper T cell levels are high, the number of regulatory T cells is allowed to flourish in order to maintain control of the growing helper T cell population. Once the antigen is removed from the system (day 5 in Fig. 6), this death rate levels out at its maximum. This new constant death rate combined with the removal of antigen stimulus serves to gradually bring the system into homeostasis. This variable death rate contributes to the dynamics in two ways. First, it allows the growth rate of the Tregs to be high enough to control the helper cells during the reaction. It allows the death rate to be high enough after the reaction to return back to normal levels of between 5 and 10 % of the helper T cell population. Second, this mechanism allows for the presence of memory helper T cells after the reaction is over.

The velocity field of the system (with $a(t) \equiv 0$) is shown in Fig. 7. The trajectories for different initial conditions are shown overlaid on the velocity field. The general tendencies toward the steady states can be seen. Of importance here is the ability of

Fig. 7 Velocity field of Eqs. (1)–(2) shown in blue. Trajectories over the velocity field are graphed for different initial conditions: $H = 0.5$, $R = 2$ (red); $H = 2.5$, $R = 3$ (green); $H = 3$, $R = 1.25$ (black) (Color figure online)



the system to cope with imbalances in the helper to regulatory ratio. In cases where regulatory T cells are too numerous (upper right portion of Fig. 7, green path), there is a markedly sharp drop in the number of regulatory T cells due to the lack of pro-inflammatory signaling. When the ratio is extended significantly in favor of helper T cells (right portion of Fig. 7 and black path), we see the gradual decrease in both the regulatory T cells and helper T cell path bringing the system to an equilibrium. If imbalances occur within a normal range (e.g., if created through antigen stimulation), we see not only a decrease in regulatory T cells, but also the transition of Tregs leads to an increase in the helper T cell population before decreasing back to equilibrium (red curve). This means that even in biologically unbalanced situations, the switching mechanism allows excess regulatory T cells to contribute to the growth of helper T cells, rather than restrict it. Biologically, this type of imbalance can occur in a number of diseases such as HIV, autoimmune diseases, and certain types of cancer (Brandt et al. 2011; Dejaco et al. 2006; Ghiringhelli et al. 2004). These diseases alter the constituency of immune cells and, therefore, the robustness of the regulatory T cell response may be an essential component in understanding the dynamics of such systems.

Functional Treg switching can contribute to the stability and to the magnitude of an immune response. Since there is little to no data on the rates at which this switching occurs, we now attempt to qualitatively determine how the value of μ should be chosen. A phase portrait of the simulations for various values of μ is shown in Fig. 8. The model with no regulatory switching ($\mu = 0$), is plotted in the dotted red line. For this model, as in the case with switching, the helper T cell population can expand and contract with respect to the regulatory T cell population. The final steady state of the system shows that the regulatory and helper T cell concentrations are on the same order of magnitude. While regulatory T cells have been shown to increase during an infection, it has also been shown that these cells return to their normal levels post pathogen exposure (Sakaguchi 2010). Hence, we consider the model with $\mu > 0$ to be more biologically relevant. Figure 8 shows that the main effect of μ is to affect the number of Tregs produced in the course of an immune response. For the values of μ shown, the maximum ratio of Treg to helpers varies from 0.09 to 0.83. Biologically, we know that the concentrations of Tregs and helper T cells should not be equal, as this would contribute to disease development. This leads us to reject the smaller

Fig. 8 Phase portrait of helper T cells versus regulatory T cells for various values of the Treg switching rate, μ

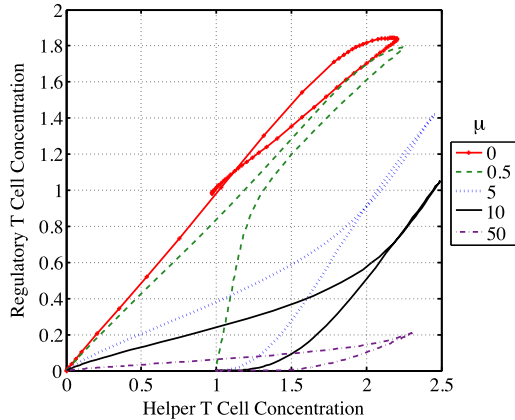
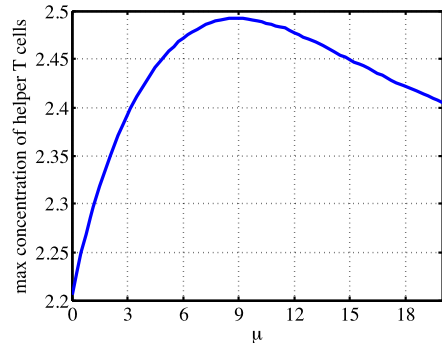


Fig. 9 Maximum helper T cell concentration as a function of the Treg switching rate, μ



μ values that lead to such ratios. We also know that in the course of an immune response, the number of Tregs should increase relative to their resting concentration (Yamazaki et al. 2003). Because these cases do not show an increase in Tregs at the infection site, we also reject $\mu = 50$ and other high values of μ , which lead to a Treg to helper T cell ratio in the range of 5–10 %.

To aid in choosing an appropriate value of μ , we consider the peak helper T cell concentration produced for certain values of μ . Figure 9 shows a graph displaying the peak helper T cell concentration as a function of μ for μ in the range [0, 20]. For these parameter values, the maximal immune response is obtained when $\mu = 9$. Consistent with what was seen in Fig. 8, Treg switching can either contribute to the strength of an immune response or diminish it. Assuming that the natural goal of the body is to maximize the strength of the immune response, this model shows that the order of magnitude of the μ parameter should be 9. Does this imply a reasonable Treg to helper T cell ratio? For the value $\mu = 9$, the maximal ratio of Tregs to helpers is 44.9 %. Though we do not have direct biological data for the value of this ratio in the periphery, as previously discussed, it should be greater than 10 % and less than 100 %. This value falls in the middle of the plausible range and hence we consider $\mu = 9$ to be an acceptable choice.

Finally, we simulate conditions that reflect the experimental setup of Duarte et al. (2009). Here, we simulate the addition of regulatory T cells into an otherwise T cell

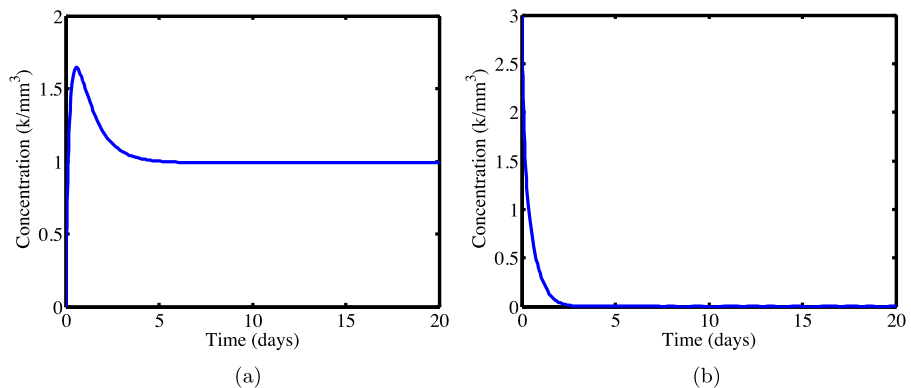


Fig. 10 Simulation of the initially helper T cell deficient system with initial conditions $H = 0$, $R = 3$. **(a)** Helper T cell deficient simulation: helper T cells. **(b)** Helper T cell deficient simulation: regulatory T cells

deficient environment. These characteristics arise when the model is considered with initial conditions $H = 0$ and $R \neq 0$. Figure 10 shows the results of this simulation. As in the experiments of Duarte et al. (2009), simulations show the regulatory T cell population decreasing due to conversion to helper T cells. In finite time, a balance of signals is achieved and the populations achieve a nonzero equilibrium. This property of the model has not been observed in past models of the cellular immune system. It shows that even in the case of extreme imbalances of initial conditions, this Treg switching mechanism serves as a stabilizing mechanism and the system is able to recover to a biologically favorable state.

4.2 Functional Switching Within the Primary Immune Response: Numerical Results and Simulations

We solve Eqs. (3)–(10) numerically using parameter values that are given in Table 2. Most parameters were obtained from Kim et al. (2010, 2012) to which we refer to for full justification. The parameters for μ and d are approximated based on their contribution to the dynamics of the system. Figure 11 shows the evolution of the APC and CTL populations. Figure 11(a) demonstrates that immature APCs are able to mature within approximately 2.5 days of the initiation of the immune response. Consistent with the biological findings of Mercado et al. (2000) and van Stipdonk et al. (2003), the entire naïve CTL population is recruited to participate in the immune response within 1 day of the start of simulation. Also, coinciding with experimental measurements showing that the T cell response peaks at 8 days after initiation (De Boer et al. 2003), the simulation also demonstrates a peak CTL response at day 8.

We consider the consequences of positive and negative signals received by CTLs in Fig. 12. Here, we study the dynamics of cytotoxic T cells relative to IL-2 and regulatory T cells. Initially, IL-2 levels rise due to the increase in the number of mature helper T cells (not shown). Further divisions past the minimal developmental program require the consumption of IL-2. Hence, as CTLs complete the minimal development program, they begin to consume IL-2, leading to a decrease in IL-2

Table 2 Estimates of parameters for model (3)–(10). Concentrations are measured in k/mm^3 . Time is measured in days. Initial conditions not explicitly given in the table are zero

	Description	Estimate	Source
$A_0(0)$	Initial concentration of immature APCs	10	Murphy et al. (2008)
$H_0(0)$	Initial concentration of naïve CD4+ T cells	0.06	Catron et al. (2004)
$K_0(0)$	Initial concentration of naïve CD8+ T cells	0.04	Catron et al. (2004)
s_A	Supply rate of immature APCs	0.3	Kim et al. (2010)
d_0	Death rate of immature APCs	0.03	Kim et al. (2010)
b	Duration of antigen availability	10	Kim et al. (2010)
c	Level of APC stimulation	2	Kim et al. (2010)
d_1	Death rate of mature APCs	0.8	Belz et al. (2007)
s_H	Supply rate of naïve CD4+ T cells	0.0018	Kim et al. (2012), Mohri et al. (2001)
s_E	Supply rate of naïve CD8+ T cells	0.0012	Kim et al. (2012), Mohri et al. (2001)
k	Kinetic coefficient	5	Kim et al. (2012)
δ_0	Death rate of naïve T cells	0.03	De Boer et al. (2003)
δ_H	Death rate of mature helper T cells	0.23	De Boer et al. (2003)
δ_K	Death rate of mature cytotoxic T cells	0.4	De Boer et al. (2003)
m_1, m_2	Number of divisions in minimal development program for helper T cells and CTLs, respectively	2, 7	Kaech and Ahmed (2001), Yang et al. (1998)
σ_H, σ_K	Duration of minimal developmental program for helper T cells and CTLs, respectively	1.46, 4	De Boer et al. (2003), Kim et al. (2012), Murphy et al. (2008)
ρ_H, ρ_K	Duration of one T cell division for helper T cells and Tregs, respectively	11/24, 1/3	De Boer et al. (2003), Kim et al. (2012), Murphy et al. (2008)
k_E	Kinetic coefficient for CTL-Treg interactions	20	Kim et al. (2012)
r_1	Rate of IL-2 secretion by mature helper T cells	10	Kim et al. (2012)
r_2	Rate of IL-2 secretion by mature CTLs	1	Kim et al. (2012)
δ_P	Decay rate of free IL-2	5.5	Kim et al. (2012)
r	Rate of differentiation of helper T cells into regulatory T cells	0.02	Kim et al. (2012), Sakaguchi et al. (2008)
d	Magnitude of dependence of Treg switching on IL-2	10	estimated empirically
μ	Rate of regulatory T cell switching	5	estimated empirically

concentration. Corresponding to the peak CTL response is the initiation of regulatory T cell proliferation. This rise in Tregs contributes to ending the CTL response in two ways. First, direct contact between Tregs and CTLs removes CTLs from the system. Second, because both Tregs and CTLs consume IL-2 as part of their division processes, competition for this cytokine reduces its availability, hindering the ability of CTLs to continue dividing.

The T cell response is also characterized in that it is robust to the precursor frequencies of antigen-specific naïve T cells. This is demonstrated by Badovinac et al. who showed that a 10,000-fold difference in antigen-specific T cell concentrations

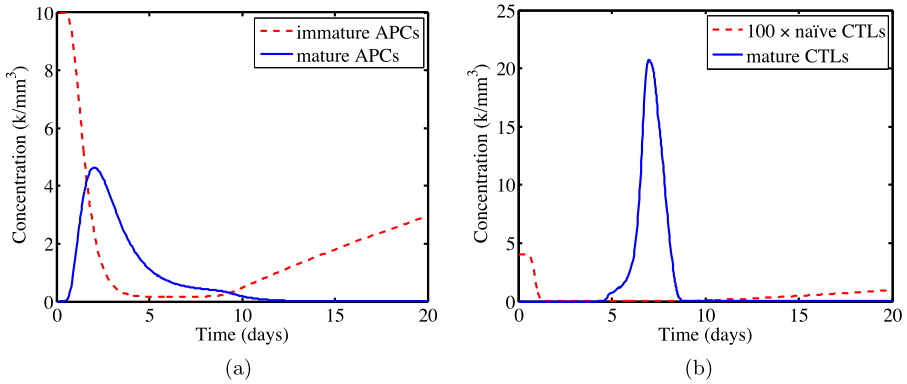


Fig. 11 Simulation of (3)–(10) with parameters given in Table 2. The time evolution of (a) antigen presenting cells and (b) cytotoxic T cells

Fig. 12 Time evolution of cytotoxic T cells, regulatory T cells and IL-2 in a simulation of (3)–(10) with parameters given in Table 2

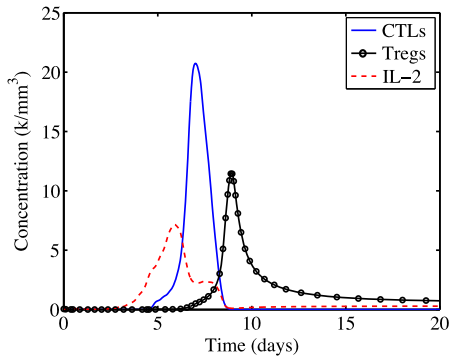
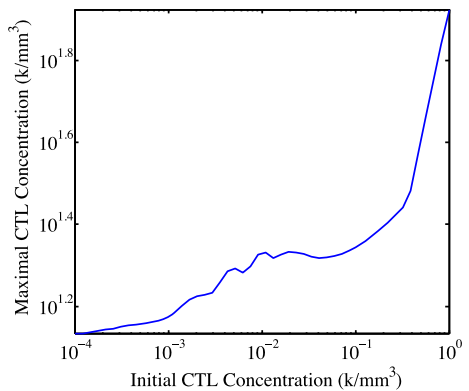


Fig. 13 Numerical experiment reflecting the biological experiments performed by Badovinac in Badovinac et al. (2007). The maximal mature CTL concentration is considered as a function of the initial naïve CTL concentration



led to a mere 13-fold difference in peak of the CD8+ T cell response (Badovinac et al. 2007). We simulate the experiments of Badovinac in Fig. 13. Here, we vary the concentration of initial naïve CTLs, $K^0(0)$, and note the maximum mature CTL concentration during the simulation. Similar to Badovinac, we perform this simula-

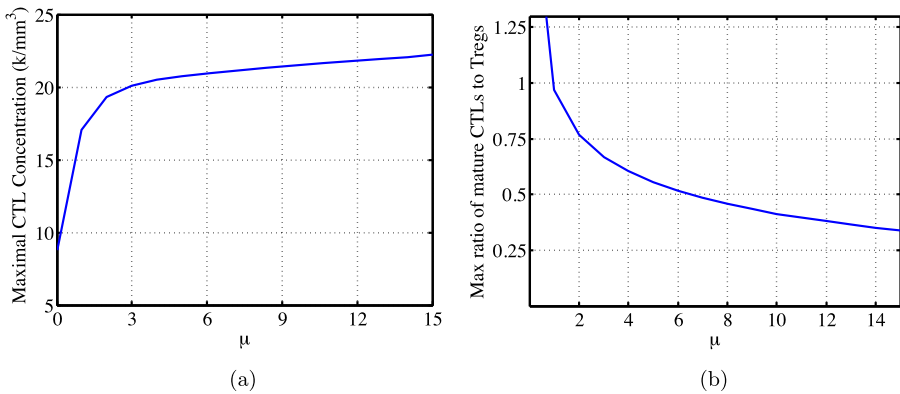


Fig. 14 (a) How does the Treg switching rate, μ , affect the maximum CTL concentration?; (b) Maximum Treg to CTL ratio

tion over a group of initial concentrations ranging over 4 orders of magnitude. Our results show that over this range of initial concentrations, there is a change in peak response that varies by approximately 1 order of magnitude. This is on par with the experimental results of Badovinac et al. (2007). This is a marked improvement over the results of Antia (2003) and Wodarz and Thomsen (2005) which display a 1 to 1 scaling of precursor frequency to peak response. When comparing the nonswitching model (Kim et al. 2012, $\mu = 0$) to the current model ($\mu = 5$), there is an improvement of this scaling from approximately 2 to 1 to the biologically supported ratio of 4 to 1. We conclude that in comparison to previous models of the immune response, the inclusion of Treg switching increases the system's robustness to initial conditions.

To study the impact of the regulatory T cell switching parameter, μ , on the dynamics of the system, we follow the techniques used to study equations (1)–(2) in Sect. 4. These results are shown in Fig. 14. In Fig. 14(a), we show the maximal number of CTLs as a function of μ . Unlike the results shown in Fig. 9, this function of μ appears to be monotonic. However, it is clear that there is a more significant change in peak CTLs per change in μ for values of μ less than 5. We also consider the maximal ratio of Tregs to CTLs when choosing a value for μ . In Fig. 14(b), we show the maximal Treg to CTL ratio as a function of μ . As discussed in Chap. 2, we expect this ratio to be in the range 0.1–1. We concentrate on ratios that lie in the center of this range and hence consider μ values between 4 and 10 (corresponding to ratios between 0.6 and 0.41).

5 Discussion

Immune regulation is an essential component of the cell mediated immune response. Failure of regulatory control of the immune system can lead to a number of complications including autoimmune diseases and immune deficiency. Despite extensive study of these topics, many immunoregulatory mechanisms are not well understood. In this work, we use mathematical models to study the dynamics of immune regulation with a focus on the role of regulatory T cell switching in the primary immune response.

Our models of immune regulation and differentiation are based on the biological study of Sakaguchi (2010). These models highlight two recently accepted biological mechanisms: active suppression of helper T cells by regulatory T cells and the transition of regulatory T cells into helper T cells. In Sect. 3.1, we included the adaptive regulation and regulatory T cell switching mechanisms in an ODE model. We demonstrated that the model displays the expected expansion/contraction dynamics and conducted a study of the effects of Treg switching. Active suppression by regulatory T cells was shown to be a plausible mechanism of immune contraction. Negative feedback provided by regulatory T cells was able to control the immune expansion and return the system to homeostasis. This simple ODE model also demonstrated that the regulatory T cell switching mechanism increases the robustness of the system, allowing for recovery from an unbalanced or even helper T cell deficient system. We characterized the affects of Treg switching on the peak magnitude of the immune response and identified the order of magnitude of the switching parameter, μ , that appears to be consistent with both the magnitude of the concentrations involved in an immune response and the ratio of Tregs to helper T cells.

Next, we extended the work presented in Sect. 3.1 concerning immune regulation and regulatory T cell switching. Here, we added a mechanism of Treg switching to the DDE model of Kim et al. (2012). The basic principles of regulatory T cell switching described in Sect. 3.1 were shown to hold within the context of this more complex model and the addition of this mechanism was shown to improve certain aspects of the immune response studied in Kim et al. (2012). An improvement of this model over past models of the immune response was seen when considering how the immune response scales with respect to initial T cell concentrations. Consistent with biological experiments, varying the initial T cell concentrations over 4 orders of magnitude led to a change in the peak immune response that varied by one order of magnitude. Also, consistent with results found in Sect. 3.1, we were also able to identify a plausible range for the rate of Treg switching, μ .

Both these models show that Treg switching may be a key contributor to immune dynamics. They motivate further experiments on the conditions and rates of the Treg switching mechanism. Such measurements could contribute to a more complete understanding of the dynamics of the contraction of the immune response.

Both of the models presented here model the response to acute infections. These models can be extended to study other occurrences such as chronic diseases or autoimmunity. It is unclear how memory cells influence the regulation of the immune system. Adding a memory T cell component is a key step in extending the applicability of our models from short-term, viral infections to chronic illnesses.

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