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Modeling regulation mechanisms in the immune system

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Abstract

We develop a mathematical framework for modeling regulatory mechanisms in the immune system. The model describes dynamics of key components of the immune network within two compartments: lymph node and tissue. We demonstrate using numerical simulations that our system can eliminate virus-infected cells, which are characterized by a tendency to increase without control (in absence of an immune response), while tolerating normal cells, which are characterized by a tendency to approach a stable equilibrium population. We experiment with different combinations of T cell reactivities that lead to effective systems and conclude that slightly self-reactive T cells can exist within the immune system and are controlled by regulatory cells.

We observe that CD8+ T cell dynamics has two phases. In the first phase, CD8+ cells remain sequestered within the lymph node during a period of proliferation. In the second phase, the CD8+ population emigrates to the tissue and destroys its target population.

We also conclude that a self-tolerant system must have a mechanism of central tolerance to ensure that self-reactive T cells are not too self-reactive. Furthermore, the effectiveness of a system depends on a balance between the reactivities of the effector and regulatory T cell populations, where the effectors are slightly more reactive than the regulatory cells.

Keywords: Immune regulation; Regulatory cells; Immune response; Immune models; Self non-self discrimination

1. Introduction

The immune system is commonly viewed as a multi-layered system in which physical barriers, an innate response, and an adaptive response are combined with a mission of protecting the body against pathogens. Each of these layers operates through an intricate system of control mechanisms whose main role is to enable the body to fight foreign pathogens in a timely fashion. Whereas innate immunity can initiate quickly, the adaptive response requires a series of signals to begin. When functioning properly, the immune response discriminates between self and non-self agents and only attacks the latter group. A dysfunctional immune system may lead to a variety of auto-immune diseases or the disadvantageous tolerance of harmful cells as in the case of chronic infections or tumors.

Jerne (1974) hypothesized that the adaptive immune system regulates itself by means of an idiotypic network. In this formulation, a first layer of clones Ab1 recognize antigen and interact with another set of clones Ab2. Then clones Ab2 interact with clones Ab3 and so on. Various papers examine immune dynamics of models based on this paradigm and show that such idiotypic networks can gain or lose tolerance to antigens and develop immunological memory from past encounters (De Boer et al., 1990; De Boer and Perelson, 1990; Varela and Stewart, 1990; Weisbuch et al., 1990). These studies focus on idiotypic networks of B cells and antibodies, but we seek to focus on the adaptive T cell response. Furthermore, these studies predate Sakaguchi's experiments (Sakaguchi et al., 1995), which established the existence of regulatory cells that primarily function by suppressing immune responses. In this paper, we apply knowledge from current immunological literature to formulate a model incorporating regulatory T cells to understand their role in regulating adaptive immune behavior.

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Adaptive immunity is broadly divided into two categories: humoral and cell-mediated immunity. Humoral immunity refers to the production of antibodies by B lymphocytes, while cell-mediated immunity refers to the production of cytotoxic T lymphocytes and activated natural killer (NK) cells. In this work, we focus on the cytotoxic T lymphocyte response of cell-mediated immunity.

The cytotoxic T lymphocyte response begins when naïve T cells are primed. Naïve T cells become primed by recognizing foreign peptides that are presented on self MHC molecules and by receiving an appropriate costimulatory signal through its CD28 receptor from antigen-presenting cells (APCs). The three major types of APCs are dendritic cells (DCs), macrophages, and B cells. DCs are the most effective APCs and are thought to stimulate most T cell responses in vivo (Janeway et al., 2005, p. 321). DCs actively consume pathogens via phagocytosis and take up extracellular particles via macropinocytosis. They present antigens on surface MHC class I and class II molecules as MHC:peptide complexes. Macrophages consume antigens via phagocytosis and present them on MHC class II molecules. B cells take up soluble antigens that bind to surface immunoglobulin molecules and also present them on MHC class II molecules. They are the least efficient of the APCs.

APCs process antigens present in lymph nodes and in the peripheral tissue. However, before effectively stimulating naïve T cells, APCs must mature. These cells mature after receiving inflammatory signals, which occur when macrophages and neutrophils release cytokines and chemokines at infection sites. After maturing, APCs migrate into lymph nodes, where they can stimulate naïve T cells.

For the most part, T lymphocyte responses begin in lymph nodes rather than in the infected tissue. Naïve T cells spend most of the time circulating through lymphoid tissue until they are stimulated. Once stimulated, they proliferate into effector cells and migrate to the infected tissue. T cells bearing the CD8+ receptor respond to MHC class I molecules and turn into cytotoxic cells, which can destroy virus-infected cells via granules that induce apoptosis. T cells bearing the CD4+ receptor respond to MHC class II molecules and differentiate into T_H1 or T_H2 cells, which can activate macrophages and B cells. In addition, CD4+ T cells can stimulate the maturation of DCs and produce positive growth signal, such as IL-2, to maintain T cell proliferation (Kasaian et al., 1991). The T lymphocyte response continues until the population of infected cells is eliminated or tolerance is reestablished.

Under normal circumstances, infections first trigger the innate immune cascade (inflammation), which sets off a chain of events that could initiate the adaptive immune response. In Sakaguchi et al. (1995) it was demonstrated that CD4+CD25+ T cells perform a key role in maintaining immunological self-tolerance. These cells make up between 3% and 15% of CD4+ cells in humans (Janeway et al., 2005; Sakaguchi et al., 1995; Taams et al., 2002; Walker et al., 2005) and originate in the thymus and periphery (Stephens et al., 2001). In this paper, we focus on the thymus-derived cells, known as naturally-occurring CD4+CD25+ regulatory cells. These cells, like CD4+CD25-T cells, are stimulated via T cell receptors (TCRs) in an antigen-specific manner (Taams et al., 2002), upon which they suppress activated immune cells in an antigen non-specific manner (Sakaguchi et al., 1995). It remains unclear how the thymus selects regulatory cells, and it was proposed in Holenbeck et al. (2001) that a portion of CD4+ cells expressing high-affinity TCR for self-peptides are selected to differentiate into regulatory cells. However, other reports (Pacholczyk et al., 2001) suggest that the TCR repertoire of regulatory cells is diverse and overlaps with the TCR repertoire of CD4+CD25-T cells.

In this model, we begin with an immune repertoire that has already been shaped by thymic selection and formulate a system of DDEs for the dynamics of key agents of the adaptive immune system within the lymph node and tissue. The delays in the equations account for the durations of T cell divisions and capture the time lag between T cells receiving stimulatory signals and completing proliferation.

We incorporate the following populations into the model: APCs, CD4+ (non-regulatory) T cells, CD8+ T cells, regulatory (CD4+CD25+) T cells, positive and negative cytokine signals, virus-infected and normal self cells, and virus and self antigens. APCs exist in either mature or immature states, and T cells can be naïve, primed, or suppressed. In addition, we model separate lymph node and tissue compartments, in which different immune populations reside and immune reactions take place at different rates.

The paper is divided into seven sections. In Section 2, we discuss general modeling assumptions as well as the specific dynamics of the immune agents. In Section 3, we explain our formulation of the system of DDEs corresponding to the dynamics discussed in Section 2. In Section 4, we discuss our estimation of parameters. Section 5 is devoted to the results and analysis. We show examples of over-, under-, and well-regulated immune responses. We also discuss the detailed dynamics of the immune response. In particular, we point out that the CD8+ T cell response occurs in two phases: lymphocyte sequestration and proliferation in the lymph node, followed by contraction and emigration to the tissue. We discuss the need for a timely transition between these two phases to result in a well-regulated immune response. We also conclude that a well-regulated response needs to have a system of central tolerance whereby the self-reactivity of the T cell repertoire is controlled, but not completely eliminated. Moreover, using Latin hypercube sampling (LHS), we observe that optimal regulation occurs if effector and regulatory cell reactivities are closely balanced with effector cells being only slightly more reactive than regulatory cells. In Section 6, we extend the discussion and deal with various stimulatory and regulatory mechanisms of the model and

their relative significances. In Section 7, we state the overall conclusion that slightly self-reactive T cells can exist in a stable immune system and are controlled by regulatory cells. In addition, we discuss implications and future directions of research, including the possible role of adaptive regulatory T cells and the possibility that immune repertoires may naturally contain mixtures of foreign-reactive effector and regulatory cells.

2. Dynamics and interactions

2.1. Modeling assumptions

In this work, we formulate a mathematical model that tracks the concentration levels of cells, molecular signals, and antigen in the tissue and lymph node compartments. Immune agents continually travel between tissue and lymph nodes, and these regions provide different environments. In particular, lymph nodes contain much higher concentrations of immune agents, allowing many more interactions than in regular tissue. Although immune agents migrate across vast networks of lymph nodes and tissue throughout the entire body, for simplicity, we model one tissue and one lymph node compartment. Furthermore, we assume that there are homogeneous environments within compartments. This further simplifies the model in that we do not have to consider the shapes of the compartments or involve spatial coordinates in our differential equations.

For convenience, we also assume we have two homogeneous populations of potential targets that represent virus-infected cells and normal self cells, and one or two homogeneous populations of each immune agent. The model can be extended to a system of more than two T cell specificities interacting with multiple populations of virus-infected cells and normal self cells with different cross-reactivities, but in this paper, we seek to understand self/non-self discrimination, so it suffices to consider two populations.

Effective self/non-self discrimination involves reacting to virus antigens and virus-infected cells while tolerating normal self cells. For simplicity and because cytotoxic responses against immune cells, called fratricide, is not well understood, we only consider non-immune cells as potential targets. Similarly, we model the antigen expressed by the target populations as one homogeneous spectrum, or combination, of peptides. In physiological settings, cells present tens of thousands of peptides and T cells decide to react or not based on this collective information (Casal et al., 2005). However, these interactions occur at a space and time scale much smaller than those of the other interactions in the model, so we consider the entire distribution of peptides expressed by the target as one collective population.

The model also incorporates APC, CD4+, CD8+, and regulatory CD4+ T cells. In addition, it includes positive and negative signals, representing cytokines that affect immune cell behavior. For clarity, in Sections 2.2–2.6, we present abridged diagrams of cell interactions. Comprehensive diagrams are shown in Appendix A.

2.2. Target cells and antigens

We assume that virus-infected cells appear in the system at a certain time (time 0) and perpetuate by increasing at a positive net growth rate. On the other hand, we assume normal self cells are supplied into the system at a constant rate and have a negative net growth rate, which causes the population to approach a stable equilibrium. We assume cells primarily produce antigen in bursts when they die, and cells die at a natural death rate and from cytotoxic interactions with effector CD8+ T cells.

Cells may reside in either of the two compartments: tissue or lymph node. We assume potential target cells reside for the most part in the tissue. Some of these cells may enter tissue-draining lymph node, but such behavior is not common and in most cases they will die quickly in the presence of a large number of immune cells. One notable exception is that cancer cells can infiltrate tumor-draining lymph nodes and disrupt proper immune function; however, in this model, we assume target cells remain in the tissue. See Fig. 1.

Inflammatory signals that accompany infections increase the flow of plasma into the tissue and the resulting drainage into the lymph node (Janeway et al., 2005, p. 321). Hence, antigen will mostly originate from cells in the tissue and drain into the lymph node, and we assume that antigen does not flow from the lymph node back to the tissue. Furthermore, immature APCs continually collect antigen in either the lymph node or periphery. See Fig. 1. Also, see Fig. A1 for a comprehensive diagram.

2.3. Antigen-presenting cells

The immune response begins at the site of infected tissue with inflammation, where inflammatory signals cause

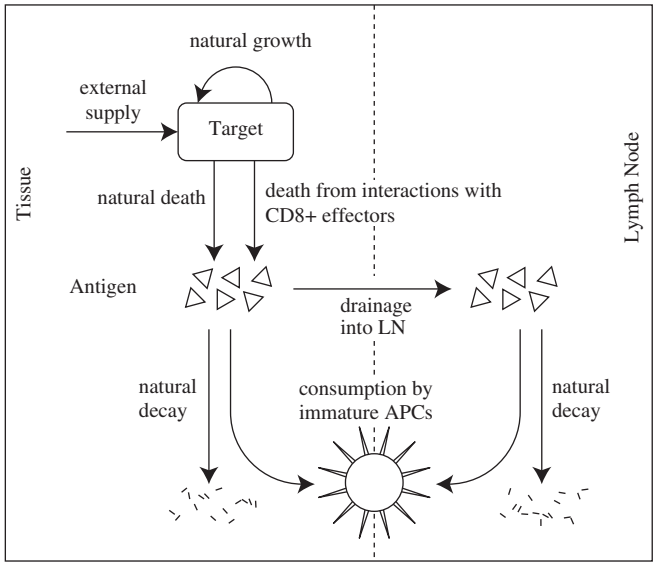

Fig. 1. Target dynamics.

APCs to mature and present antigen to T cells in the lymph node. Inflammatory signals come from several sources, including cytokines secreted by immune agents and pathogenic particles that stimulate APC receptors (Janeway et al., 2005, p. 321). Since this is a complicated process induced by the innate immune response, we simulate inflammation by increasing the rate that immature APCs are stimulated to mature in the tissue. In addition, immature APCs may be induced to mature by interactions with primed CD4+ T cells.

Since DCs are the most important APCs, especially for triggering naïve T cells, we use information about DCs to model the APCs in our system. When they are immature, DCs pick up antigen, express low levels of MHC II and costimulatory molecules, and cannot stimulate naïve T cells (Wilson et al., 2004; Janeway et al., 2005, p. 331). Instead, immature DCs induce tolerance by rendering T cells anergic (Kubach et al., 2005; Janeway et al., 2005, Sections 8–10). Upon maturation, DCs upregulate expression of costimulatory molecules and stimulate naïve T cells. At this point, they efficiently process and present antigens captured before or at time of maturation, but for the most part stop acquiring new antigens (Wilson et al., 2004; Janeway et al., 2005, p. 331). Maturation induces DCs to detach from the peripheral tissue and travel to a lymph node (Janeway et al., 2005, p. 331).

Less is known about the migration of immature APCs, but we assume that new immature APCs are supplied by bone marrow stem cells at a constant rate into the lymph node and migrate to the tissue at a fixed rate. This assumption ensures a continuously replenished population of immature APCs in the lymph node and tissue compartments. In steady state, most APCs in lymph nodes are immature (Wilson et al., 2003). However, such APCs may pick up antigens that drain into the lymph node and be stimulated by interacting with primed CD4+ T cells.

APCs continually process and replace surface antigen, and the typical turnover rate for immature APCs is faster than that for mature APCs. We will estimate these rates in Section 4. In our model, we are only concerned with the antigens produced and presented by target cells, since these are the only populations the T cells will attack. Hence, we assume the following:

1. Immature APCs capture new antigen at a certain rate, while mature APCs do not (Wilson et al., 2004; Janeway et al., 2005, p. 331).
2. APCs lose surface antigen at a certain rate due to turnover (Wilson et al., 2004).
3. Immature APCs mature at a proportional rate that depends on the inflammation level.
4. The motion of APCs is directed. Mature APCs travel from the tissue to the lymph node (Janeway et al., 2005, p. 331). Immature APCs travel from the lymph node to the tissue.
5. Immature APCs are supplied at a constant rate into the lymph node and then travel to the tissue.

APCs may also be acted upon by regulatory cells, but we postpone the discussion of regulatory mechanisms until Section 2.6. Fig. 2 describes the dynamics of immature and mature APCs. Also, see Fig. A2 for a comprehensive diagram of APC interactions.

2.4. T cells

DCs present both MHC I and II molecules, and thus can prime both CD4+ and CD8+ T cells. Since DCs are responsible for most, if not all, of the stimulation of T cells, the APCs in our model, like DCs, can prime both CD4+ and CD8+ T cells, and naïve T cells get primed when they recognize appropriate antigen on surfaces of mature APCs. Alternatively, naïve T cells become anergic after interacting with immature APCs. The role of anergic cells is unclear, so for convenience, we assume anergy is an inactive, terminal state and model anergic cells as effectively dead.

Once a CD4+ T cell is primed, it proliferates and turns into an effector that causes APCs to mature and secretes positive growth signal such as IL-2 (Kasaian et al., 1991). Positive growth signals stimulate both CD4+ and CD8+ cells to divide. Since CD4+ cells are more readily stimulated than CD8+ cells and produce growth signal much more efficiently (Janeway et al., 2005, Sections 8–14), we do not model this autocrine loop and instead assume that CD4+ cells are self-sufficient to divide upon further stimulation by mature APCs. The assumption that CD4+ cell division depends on repeated stimulation by mature APCs is based on experimental observations that removal of antigen early in an immune response diminishes CD4+ expansion, but not CD8+ expansion (Mercado et al., 2000).

Naïve CD4+ and CD8+ T cells continuously circulate through lymph nodes, back into the bloodstream, and into other lymph nodes where they interact with thousands of APCs every day (Janeway et al., 2005, p. 323). However, in our model, since we have only one lymph node and one tissue compartment, we assume that naïve and pre-primed T cells reside in the lymph node until they are fully primed. In addition, we assume that naïve T cells are supplied by stem cells at a constant rate into the lymph node. See Fig. 3 for a diagram of CD4+ dynamics.

For a CD8+ cell to be primed it has to be stimulated by mature APCs and receive a positive growth signal (Janeway et al., 2005, Sections 8–14). We account for this two-step signaling by shifting naïve CD8+ T cells to a pre-primed (IL-2 receptor positive) state after stimulation from APCs. If they further receive a positive growth signal while in the pre-primed state, they proliferate and progress to effector cells. If they do not receive a positive growth signal for a certain amount of time, they return to the naïve state.

Experiments have shown that CD8+ cells in vivo can initiate division even in CD4-depleted mice (Kasaian et al., 1991). Additional studies have shown that upon stimulation, CD8+ cells can begin IL-2 production to drive initial expansion, but this response is short-lived because these

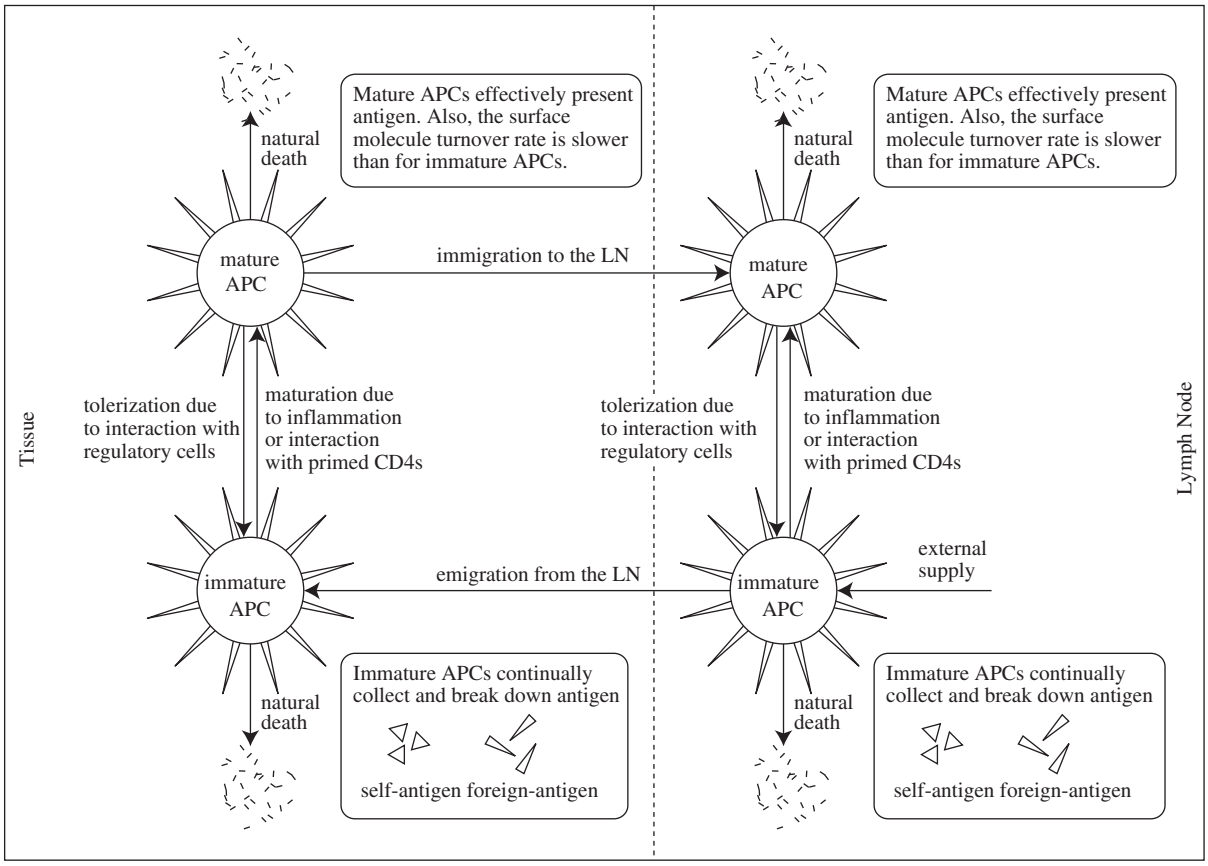

Fig. 2. APC interactions.

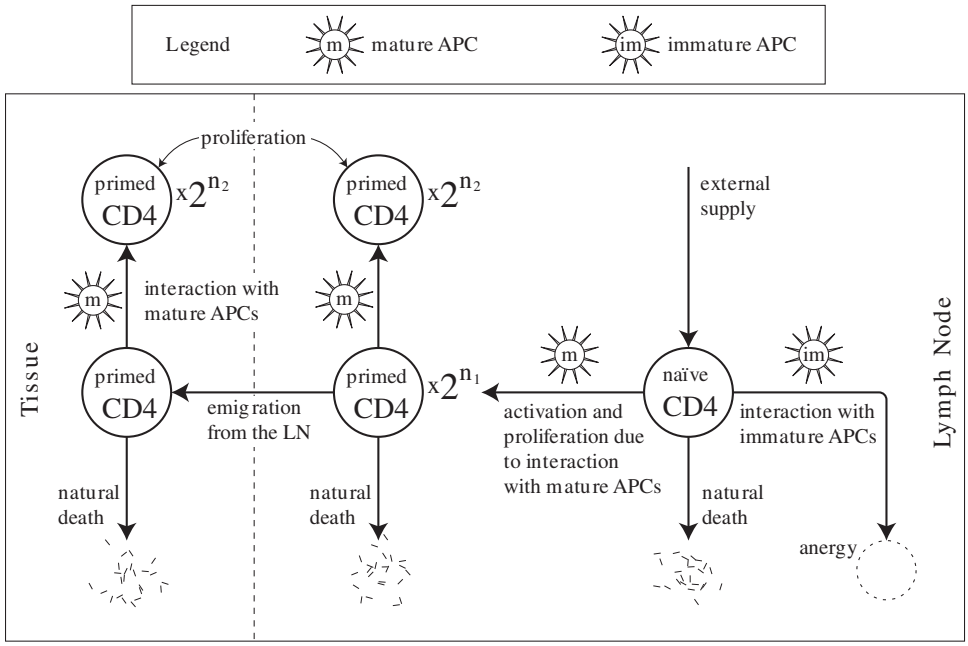

Fig. 3. CD4+ T cells.

cells enter an activation-induced non-responsive (AINR) state (Deeths et al., 1999; Tham and Mescher, 2001, 2002; Tham et al., 2002). CD8+ cells become AINR within three days of stimulation and no longer produce IL-2, but are still able to divide in response to exogenous IL-2 and respond cytotoxically to target cells (Deeths et al., 1999;

Tham and Mescher, 2001). The presence of exogenous IL-2 allows prolonged expansion of AINR CD8+ cells but only marginally affects their ability to resume production of IL-2 (Uhlin et al., 2005). Thus, the initial clonal expansion of CD8+ cells may be helper-independent, but quickly progresses to a helper-dependent stage.

We account for this phenomena by modeling that pre-primed CD8+ cells secrete IL-2, causing these cells to enter the initial phase of proliferation, consisting of about $n = 8$ divisions. In our model, we consider this initial proliferation as one process and do not separate it into individual divisions and interactions. Furthermore, the whole process takes approximately 4–5 days, meaning that the newly multiplied CD8+ T cells have entered the AINR state and no longer secrete IL-2.

Effector CD8+ T cells are cytotoxic and can kill target cells upon interaction. After priming, CD8+ cells will divide upon further interaction with positive growth signals. We base this assumption on the experimental evidence that CD4+ cells are the primary IL-2 producers, while CD8+ cells are the primary proliferators (Kasaian et al., 1991), and CD8+ cells are able to undergo antigen-independent proliferation after activation (Antia et al., 2003; De Boer et al., 2003).

As before, naïve CD8+ T cells that interact with immature APCs presenting the appropriate antigen may be rendered anergic (Kubach et al., 2005; Janeway et al., 2005, Sections 8–10). See Fig. 4 for a diagram of CD8+ dynamics.

Primed CD8+ cells need continual stimulation from antigen or mitogen to continue dividing (Janeway et al., 2005). IL-2 stimulation is not enough. Hence, we assume that primed CD8+ T cells that are not occupied with division may drop to a non-proliferating, effector state at a constant rate. These non-proliferating CD8+ T cells possess the same cytotoxic capabilities as primed and proliferating effector cells, but they no longer divide in response to positive growth signals. Non-proliferating effector T cells may be restimulated into the primed and proliferating state by interaction with mature APCs. This modeling hypothesis is described in Fig. 5.

Like APCs, primed T cells are also affected by regulatory T cells, but we postpone the discussion of regulatory interactions until Section 2.6. In this model, we are mostly interested in the primary immune response, so for simplicity we do not model memory T cells. See Fig. A3 for a comprehensive diagram of T cell interactions.

2.5. Molecular signals

The behavior of immune agents is affected by cytokines, such as IL-2 which promotes T cell growth, or IL-10 and TGF-β which suppress their activity. Many different cytokines interact with immune agents, but in this model, we categorize all cytokines by two groups: positive and negative signals.

As mentioned in Section 1, primed T cells secrete a positive growth signal, such as IL-2, which promote T cell division (Janeway et al., 2005). Regulatory cells (CD4+CD25+) release cytokines transmitting negative signals. The regulatory role of these cytokines in vivo is not fully known; however, interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) function primarily as immunosuppressors (Janeway et al., 2005, p. 627). The cytokine IL-10 blocks the production of IL-2, TNF-α, and IL-5 and hinders antigen-presentation by reducing the expression of MHC and co-stimulatory molecules by APCs (Janeway et al., 2005). TGF-β also blocks cytokine

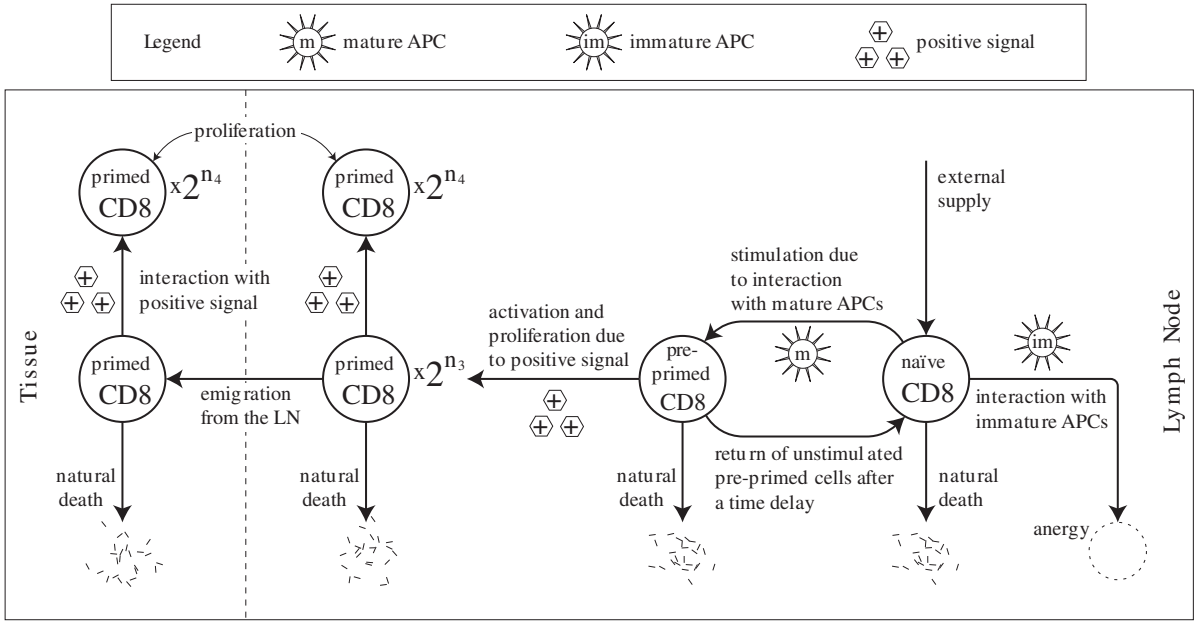

Fig. 4. CD8+ T cells.

production and hinders cell division and cytotoxic responses (Janeway et al., 2005).

Cytokines are released by other cells, especially APCs, but most cytokines express both positive and negative effects depending on the cells they interact with and the context of other cytokines. Due to the ambiguity and complexity of the roles of cytokines, we model that only primed CD4+ T cells and regulatory CD4+ T cells release positive and negative signals, respectively. Furthermore, we model that CD8+ cells consume positive signal, which stimulates their division, and any circulating signal that is not consumed decays at a constant rate. See Fig. 6. Also, see Fig. A3 for a comprehensive diagram of interactions between molecular signals and T cells.

2.6. Regulatory mechanisms

We focus on the regulatory mechanism of CD4+CD25+ cells. Although there are other types of regulatory CD4+ cells, such as T_R1 and T_R3, and there may be regulatory CD8+ cells (Jiang and Chess, 2004), CD4+CD25+ cells play a key role (Sakaguchi et al., 1995), and small numbers can help maintain self tolerance (Jiang and Chess, 2004).

The experiments of Sakaguchi et al. (1995) show that under normal circumstances, potentially self-reactive CD4+ T cells are present in the tissue and their activation and proliferation is controlled by the presence of CD4+CD25+ cells. These cells are distinguished from CD4+ effector cells by high level, persistent expression of the IL-2 receptor alpha chain (CD25) (Sakaguchi et al., 1995).

These cells represent between 3% and 15% of the CD4+ T cells in human circulation and seem to be positively selected in the thymus based on high-affinity to self-peptides (Janeway et al., 2005; Walker et al., 2005). In addition, they are activated like other T cells by stimulation via their TCR (Janeway et al., 2005, p. 627). Once activated, they regulate the immune system by suppressing the immune response to self and non-self antigens in an antigen non-specific manner (Janeway et al., 2005; Sakaguchi et al., 1995). Removing them increases reactivity to both non-self and self antigens, resulting in autoimmunity (Sakaguchi et al., 1995).

For the most part, regulatory CD4+ cells are thought to function as part of the innate regulatory response (Jiang and Chess, 2004). For example, it is known that regulatory CD4+ cells can function during a primary immune response (Jiang and Chess, 2004). This observation suggests that regulatory CD4+ cells always make up a fraction of the immune cell population and are ready to respond in a suppressive capacity.

These regulatory cells are positively selected in the thymus based on high affinity to self-peptides (Janeway et al., 2005). Like other T cells, they are initially naïve and are activated by receiving stimulation from mature APCs (Janeway et al., 2005). Hence, we assume that naturally-occurring regulatory cells are supplied at a constant rate and are stimulated in the same way as non-regulatory CD4+ cells. See Fig. 7.

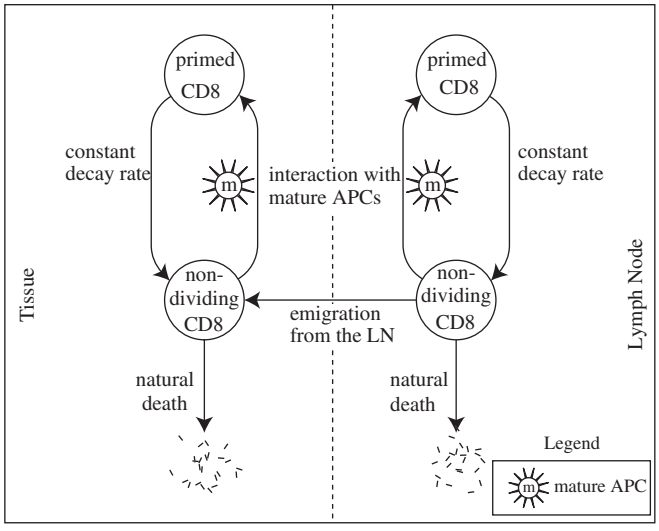

Fig. 5. Scenario 1: Non-proliferating CD8+ T cells. In Scenario 2: Primed CD8+ T cells never drop into a non-proliferating state.

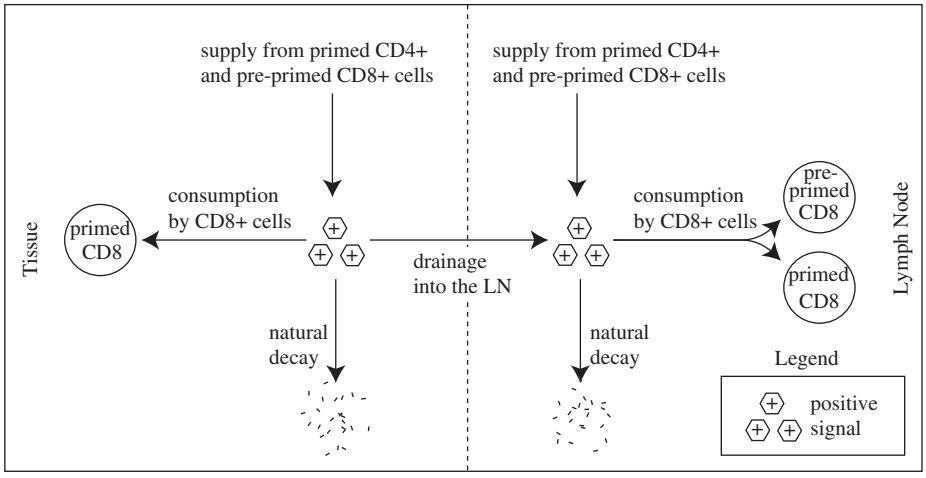

Fig. 6. Positive growth signal.

We propose three possible means by which regulatory CD4+ cells may regulate immune responses. From the experiments in Sakaguchi et al. (1995), the primary regulatory function of CD4+CD25+ cells seems to be to suppress the activity of primed CD4+ T cells. In addition, the in vitro regulatory capacity of these cells is observed to be contact dependent and not only due to cytokines (Janeway et al., 2005, p. 627; Jiang and Chess, 2004). As an extension, we suppose regulatory cells also suppress primed CD8+ cells.

Also, since regulatory CD4+ cells express the high affinity IL-2 receptor molecule CD25, we propose that regulatory CD4+ cells consume IL-2 and other positive growth signals. In addition, studies have shown that regulatory CD8+ cells can render DCs tolerogenic (Chang et al., 2002). As an extension, it seems reasonable to suppose that regulatory CD4+ cells also interact with DCs, since we know that non-regulatory CD4+ T cells interact with DCs. For example, primed CD4+ T cells cause DCs to mature. Thus, in our model, we assume that regulatory CD4+ cells may interact with APCs, in general, to render them tolerogenic. Furthermore, since interactions between CD4+ T cells and APCs are mostly contact dependent, we assume the same for regulatory CD4+ cells.

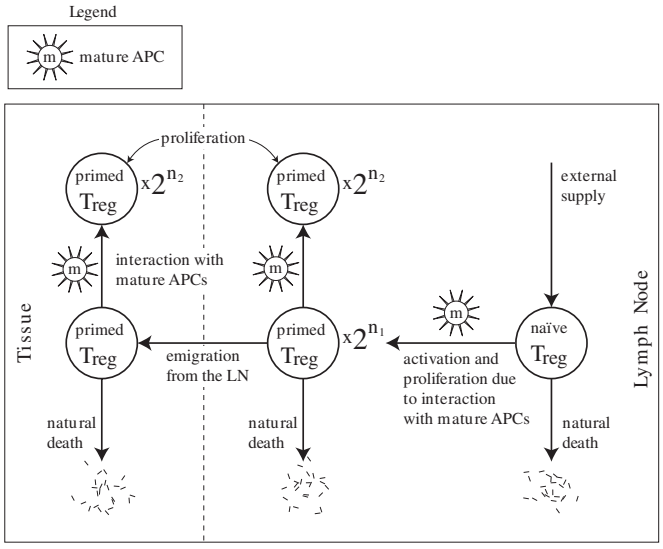

Fig. 7. Naturally-occurring regulatory CD4+ cells.

Beyond contact dependent regulatory mechanisms, CD4+CD25+ cells also release cytokines as discussed in Section 2.5. We assume that a negative signal decreases the secretion rates $s_{x,1}$ and $s_{x,2}$ of positive signals by primed CD4+ and CD8+ cells, decreases the probability $p_{PK/x}$ that primed CD8+ cells divide in response to stimulus from positive growth signal, decreases the probability $p_{PK/T}$ that a primed CD8+ cell kills its target, and decreases the probabilities $p_{TC/B}$ and $p_{TC/B}$ that CD4+ cells react to mature APCs. We assume these rates and probabilities decrease exponentially at rate c as a function of the concentration of negative signals. These probabilities and rates will be discussed in more detail in Section 3.

Hence, we have the following five mechanisms (shown in Fig. 8):

1. Regulatory CD4+ cells suppress primed CD4+ T cells in an antigen non-specific manner via direct cell-to-cell interactions. They cause primed CD4+ T cells to become unresponsive.
2. Regulatory CD4+ cells suppress primed CD8+ T cells in an antigen non-specific manner via direct cell-to-cell

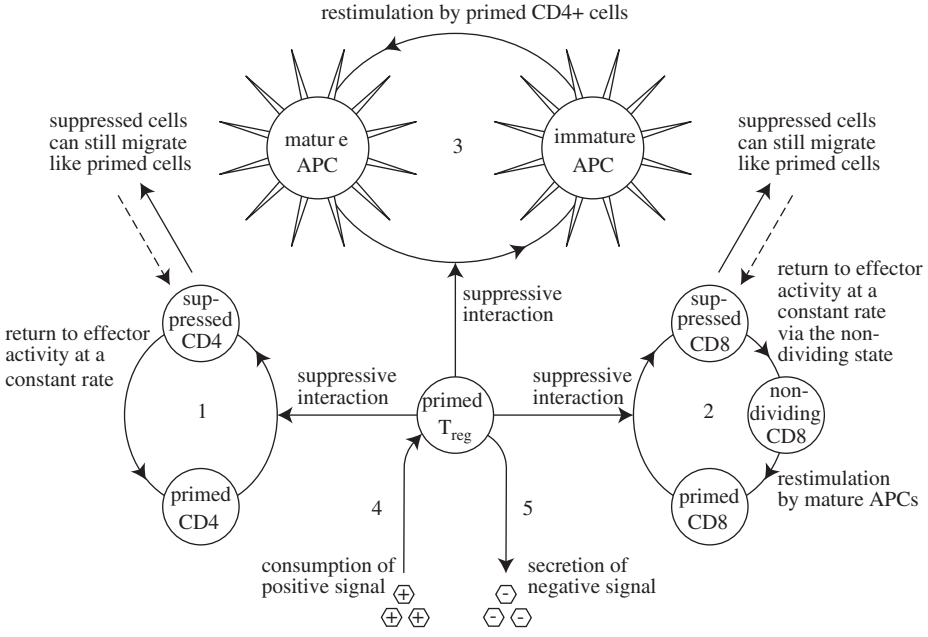

Fig. 8. Suppressive mechanisms of regulatory cells.

interactions. They cause primed CD8+ T cells to become unresponsive.
3. Regulatory cells render APCs tolerogenic. They do this via direct cell-to-cell interaction.
4. Regulatory cells may control CD8+ effector proliferation by competing for positive growth signal.
5. Regulatory cells suppress immune agents by secreting negative signals.

3. A mathematical model

In this section, we formulate a mathematical model for the dynamics and interactions that were described in Section 2. We write the model as a system of DDEs. The variables denoting each population are defined in this section and listed again in Table E1.

3.1. Target cells and antigen

The equations governing the populations of normal and virus-infected cells T_1^T and T_2^T are

$$\frac{dT^T}{dt} = s_T + (g_T - d_T)T^T - k(p_{T/PK}\phi(y^T)(P_K^T + Q_K^T))T^T, \tag{3.1}$$

where T^T is the 2×1 vector $[T_1^T, T_2^T]^t$. Likewise, the terms P_K^T and Q_K^T are also vectors $[P_{K,1}^T, P_{K,2}^T]^t$ and $[Q_{K,1}^L, Q_{K,2}^L]^t$ corresponding to the "foreign-reactive" and "self-reactive" sets of T cell populations. From now on, all variables denoting target, antigen, or T cell populations are 2×1 vectors. The superscript "T" indicates that we are considering the population in the tissue as opposed to the lymph node. We assume target cells normally exist in the tissue only.

The term s_T denotes the constant supply rate of cells into the tissue. The terms g_T and d_T denote the constant growth and death rates, respectively. For more generality between the two populations, we also consider these constants as 2×1 vectors and interpret the product of two 2×1 vectors as a componentwise product.

The term $p_{T/PK}$ denotes the probability that a target cell gets killed by an interaction with an effector CD8+ T cell. It is a 2×2 diagonal matrix whose upper left entry denotes the probability that an effector CD8+ cell from population 1 (the self-reactive population) destroys target 1 (the self population). The lower right entry denotes the probability that an effector CD8+ cell from population 2 (the foreign-reactive population) destroys target 2 (the foreign population). Note that the off-diagonal entries of $p_{T/PK}$ can denote cross-reactivities between the two T cell and two target populations, but for this study, we set all cross-reactivities to 0.

We assume the law of mass action, which states that the rate of interaction between two populations is proportional to the product of their concentrations. Hence, the expression $k(P_K^T + Q_K^T)T^T$ is the rate of interaction between target cells and effector CD8+ T cells in the tissue. The values of all the parameters are discussed in Section 4.

As mentioned in Section 2.6, we assume that certain rates of interaction decrease exponentially with respect to the concentration of negative signal at rate c. To account for the effect of negative signaling (regulatory mechanism 5), we adjust the rate of interaction between target cells and CD8+ T cells by the factor $\phi(y^T)$, where $\phi(y^T) = e^{-cy^T}$.

We also model antigen populations: $\alpha^L = [\alpha_1^L, \alpha_2^L]^t$ for the lymph node, and $\alpha^T = [\alpha_1^T, \alpha_2^T]^t$ for the tissue. (The superscript "L" indicates a population in the lymph node.) The equations governing the antigen concentrations in the lymph node and tissue are

$$\frac{d\alpha^L}{dt} = f\alpha^T + -d_\alpha\alpha^L - k\sum_{i,j=0}^{1} A_{ij}^L\alpha^L, \tag{3.2}$$

$$\frac{d\alpha^T}{dt} = -\frac{f\alpha^T}{V} + s_\alpha(d_T + k(p_{T/PK}\phi(y^T)(P_K^T + Q_K^T)))T^T - d_\alpha\alpha^T - k\sum_{i,j=0}^{1} A_{ij}^T\alpha^T. \tag{3.3}$$

The first terms in (3.2) and (3.3) account for the drainage of antigen from the tissue into the lymph node at rate f. Since we are dealing with population concentrations, we adjust the flow rates by the constant V, which denotes the ratio of the volume of the tissue compartment to the volume of the lymph node compartment.

The second term in (3.3) corresponds to the supply of antigen from target dying in the tissue. We assume that antigen is released at a rate proportional to the death rate of its corresponding cell population, where the proportionality constant is s_α. The penultimate terms in both equations correspond to the natural decay of circulating antigen at rate d_α. The final terms correspond to the rates at which antigen is taken up by immature APCs, A_{ij}^L and A_{ij}^T. For simplicity, we assume that any antigen that interacts with an immature APC is taken up. The constant k is the mixing coefficient as in (3.1). The subscripts i and j take values 0 or 1 depending on whether the APC is presenting antigens 1 and/or 2, respectively.

3.2. CD4+ T cells

The equation governing the naïve CD4+ T cell population N_H^L is

$$\frac{dN_H^L}{dt} = \omega s_H - d_{NH}N_H^L - k\mathcal{J}(p_{TC/A}, A_{ij}^L)N_H^L - k\mathcal{J}(p_{TC/B}\phi(y^L), B_{ij}^L)N_H^L. \tag{3.4}$$

As before N_H^L is a 2×1 vector representing the two T cell populations: the self-reactive T cell population and the foreign-reactive T cell population. The coefficients s_H and ω denote the supply rate and frequency of antigen-specific

T cells, and d_{NH} denotes the proportional death rate of naïve CD4+ T cells. As a simplifying assumption, we assume that the supply rate is the same for both CD4+ populations, and that the death rate is the same for both CD4+ populations. Thus, we take s_H and d_{NH} to be scalars rather than vectors. From now on, we apply this assumption to the supply and death rates for all T cell populations.

The third and fourth terms of (3.4) correspond to the rate at which naïve CD4+ cells interact with immature APCs, A^L_{ij}, and mature APCs, B^L_{ij}, respectively. The fourth term contains the adjustment factor $\phi(y^L)$ due to the negative signal in the lymph node. The parameter $p_{\mathrm{TC/A}}$ ($p_{\mathrm{TC/B}}$) is a 2×2 matrix (a_{ij}) denoting the probabilities that T cell i reacts to an immature (mature) APC presenting antigen j. As shown in Fig. 3, such interactions result in T cell anergy and stimulation, respectively.

The function $\mathscr{J}$ is defined by

$$\mathscr{J}(p, A_{ij}) = p\begin{bmatrix} A_{10} \\ A_{01} \end{bmatrix} + \begin{bmatrix} 1-(1-p_{11})(1-p_{12}) \\ 1-(1-p_{21})(1-p_{22}) \end{bmatrix} A_{11}, \quad (3.5)$$

where p is a 2×2 matrix and A_{ij} is the collection of APC populations A_{00}, A_{10}, A_{01}, and A_{11}. The function maps to a 2×1 vector denoting the rates at which both CD4+ cell populations react to immature APCs. The first term in (3.5) is the rates at which CD4+ cells interact with APCs carrying antigens 1 or 2 exclusively, and the second term is the rates at which CD4+ cells interact with APCs carrying both antigens 1 and 2 simultaneously. As before, the products of two 2×1 vectors are interpreted as a componentwise products. The function also applies for mature APCs, B_{ij}.

The equations for primed CD4+ T cell populations P^L_H and P^T_H in the lymph node and tissue are

$$\begin{aligned}\frac{\mathrm{d}P^L_H}{\mathrm{d}t} = & -\tilde{f}P^L_H - d_{PH}P^L_H + 2^{n_{NH}}k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^L), \\ & B^L_{ij}(t-n_{NH}\tau))N^L_H(t-n_{NH}\tau) \\ & - k\mathscr{J}(p_{\mathrm{TC/B}}, B^L_{ij})P^L_H + 2^{n_{PH}}k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^L(t-n_{PH}\tau)), \\ & B^L_{ij}(t-n_{PH}\tau))P^L_H(t-n_{PH}\tau) \\ & - p_{\mathrm{TC/R}}kP^L_RP^L_H + r_uS^L_H, \end{aligned} \quad (3.6)$$

$$\begin{aligned}\frac{\mathrm{d}P^T_H}{\mathrm{d}t} = & \frac{\tilde{f}P^L_H}{V} - d_{PH}P^T_H - k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^T), B^T_{ij})P^T_H \\ & + 2^{n_{PH}}k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^T(t-n_{PH}\tau)), \\ & B^T_{ij}(t-n_{PH}\tau))P^T_H(t-n_{PH}\tau) \\ & - p_{\mathrm{TC/R}}kP^T_RP^T_H + r_uS^T_H. \end{aligned} \quad (3.7)$$

The first term of (3.6) and (3.7) corresponds to the flow of primed CD4+ T cells out of the lymph node. The coefficient $\tilde{f}$ denotes the rate at which T cells flow from the lymph node into the tissue. In the second terms, the coefficient d_{PH} is the death rate of primed CD4+ T cells.

The third term in (3.6) accounts for the proliferation of naïve CD4+ T cells due to stimulation by mature APCs. It corresponds to the final term of (3.4). The exponent n_{NH} is the average number of divisions taken by naïve CD4+ T cells upon stimulation, and the time delay τ is the average amount of time it takes to complete one cell division cycle. Hence, newly proliferated CD4+ cells appear $n_{NH}\tau$ time units after the initial stimulation.

We also use the parameter $p_{\mathrm{TC/B}}$ for probabilities that primed CD4+ cells are stimulated by mature APCs. In addition, n_{PH} is the average number of divisions taken by primed CD4+ T cells upon stimulation. Hence, the fourth to last terms of (3.6) and (3.7) are the rates at which primed CD4+ cells are stimulated by interactions with mature APCs. The third to last terms account for the proliferation of these CD4+ cells. These dynamics are also outlined in Fig. 3. Due to negative signaling, all terms involving stimulatory interactions between CD4+ T cells and APCs are adjusted by the factor $\phi(y)$ or the delayed version, $\phi(y(t-n_{PH}\tau))$.

The final two terms of each equation account for regulatory mechanism 1 in Fig. 8. The variables P^L_R and P^T_R are the concentrations of primed regulatory cells in the lymph node and tissue, respectively. The coefficient $p_{\mathrm{TC/R}}$ is the probability that interactions between a regulatory cell and a primed T cell causes the T cell to become suppressed. Suppressive interactions between regulatory cells and T cells are not antigen-specific (i.e., there is no distinction between T cell populations 1 and 2), so $p_{\mathrm{TC/R}}$ is a scalar. The coefficient r_u is the proportional rate that suppressed CD4+ cells, S^L_H and S^T_H, become unsuppressed.

3.3. Suppressed CD4+ T cells

The equations for the concentrations of suppressed CD4+ cells S^L_H and S^T_H are

$$\frac{\mathrm{d}S^L_H}{\mathrm{d}t} = -\tilde{f}S^L_H - d_{SH}S^L_H + p_{\mathrm{TC/R}}kP^L_RP^L_H - r_uS^L_H, \quad (3.8)$$

$$\frac{\mathrm{d}S^T_H}{\mathrm{d}t} = \frac{\tilde{f}S^L_H}{V} - d_{SH}S^T_H + p_{\mathrm{TC/R}}kP^T_RP^T_H - r_uS^T_H. \quad (3.9)$$

The first two terms of Eqs. (3.8) and (3.9) account for the flow of suppressed CD4+ cells out of the lymph node and their natural death rate d_{SH}. The final two terms of both equations are the negatives of the final two terms in (3.6) and (3.7). They correspond to the suppression of CD4+ cells by regulatory cells and the natural rate of return to effector activity. See regulatory mechanism #1 in Fig. 8.

3.4. CD8+ T cells

The equations governing the naïve CD8+ T cell population N^L_K and the pre-primed (IL-2 receptor positive)

CD8+ T cell population O_K^L are

$$\begin{aligned}\frac{dN_K^L}{dt} &= \omega s_K - d_{NK}N_K^L - k\mathcal{J}(p_{TC/A}, A_{ij}^L)N_K^L \\ &\quad - k\mathcal{J}(p_{TC/B}\phi(y^L), B_{ij}^L)N_K^L \\ &\quad + Dk\mathcal{J}(p_{TC/B}\phi(y^L(t-\sigma)), B_{ij}^L(t-\sigma))N_K^L(t-\sigma),\end{aligned} \tag{3.10}$$

$$\begin{aligned}\frac{dO_K^L}{dt} = &- d_{NK}O_K^L - p_{PK/x}\phi(y^L)kx^LO_K^L \\ &+ k\mathcal{J}(p_{TC/B}\phi(y^L), B_{ij}^L)N_K^L \\ &- Dk\mathcal{J}(p_{TC/B}\phi(y^L(t-\sigma)), \\ &B_{ij}^L(t-\sigma))N_K^L(t-\sigma).\end{aligned} \tag{3.11}$$

In Eq. (3.10) for naïve CD8+ cells, the parameters s_K, ω, and d_{NK} are the supply rate, frequency of antigen-specific T cells, and the death rate of naïve CD8+ cells in the lymph node. The third and fourth terms of (3.10) are the rates of interaction with immature and mature APCs. They correspond to the third and fourth terms of (3.4) for naïve CD4+ cells. In addition, the function $\mathcal{J}$ is as defined in (3.5). The 2×2 matrix $p_{TC/A}$ ($p_{TC/B}$) corresponds to the probabilities that naïve CD8+ T cells react to an encounter with immature (mature) APCs. As shown in Fig. 4, this interaction results in anergy (stimulation). Again, all stimulatory interactions are adjusted by the exponential decay function due to negative signaling.

The final term of (3.10) is the rate that pre-primed CD8+ cells return to the naïve state if they remain unstimulated by a positive growth signal for a time delay of σ. Since not every cell that enters the pre-primed state returns to the naïve state, the discounting factor D accounts for the loss of pre-primed cells due to natural death or promotion to the effector state.

We assume that pre-primed CD8+ T cells die at the same rate as naïve CD8+ T cells, so the first term in (3.11) is the natural death rate of pre-primed CD8+ cells. The second term corresponds to the rate that pre-primed CD8+ T cells O_K^L are stimulated by a positive growth factor x^L. Once stimulated, these cells leave the pre-primed state and become fully primed effectors. We assume that pre-primed CD8+ T cells are stimulated at the same rate $p_{PK/x}$ as primed CD8+ T cells. The third term is the rate that naïve cells are stimulated into the pre-primed state by mature APCs. It is the negative of the fourth term in (3.4) for naïve cells. The fourth term is the rate that unstimulated pre-primed cells return to the naïve state and is the negative of the fifth term in (3.4).

The delay σ denotes the length of time pre-primed T cells wait to receive a positive growth signal before returning to the naïve state, and the discounting factor

$$\begin{aligned}D &= \exp\left\{-\int_{t-\sigma}^{t}(d_{NK} + p_{PK/x}\phi(y^L)kx^L)\right\} \\ &= e^{-d_{NK}\sigma}\exp\left\{-p_{PK/x}k\int_{t-\sigma}^{t}\phi(y^L)x^L\right\}\end{aligned} \tag{3.12}$$

accounts for the decrease in population of pre-primed T cells over this time interval. See Appendix B for the derivation of (3.12).

Note that the addition of the discounting factor D turns (3.10) and (3.11) into integro-DDEs, but we can turn these into first-order DDEs by adding the equation

$$\frac{dD}{dt} = -p_{PK/x}k(\phi(y^L)x^L(t) - \phi(y^L(t-\sigma))x^L(t-\sigma))D, \tag{3.13}$$

with an initial condition $D(0) = e^{-d_{NK}\sigma}$.

The equations for primed CD8+ T cell populations P_K^L and P_K^T in the lymph node and in the tissue are

$$\begin{aligned}\frac{dP_K^L}{dt} = &- \tilde{f}P_K^L - d_{PK}P_K^L + 2^{n_{OK}}p_{PK/x}\phi(y^L(t-n_{OK}\tau)) \\ &\times kx^L(t-n_{OK}\tau)O_K^L(t-n_{OK}\tau) \\ &- p_{PK/x}\phi(y^L)kx^LP_K^L + 2^{n_{PK}}p_{PK/x}\phi(y^L(t-n_{PK}\tau)) \\ &\times kx^L(t-n_{PK}\tau)P_K^L(t-n_{PK}\tau) \\ &- r_rP_K^L + k\mathcal{J}(p_{TC/B}\phi(y^L), B_{ij}^L)Q_K^L \\ &- p_{TC/R}kP_R^LP_K^L + r_uS_K^L,\end{aligned} \tag{3.14}$$

$$\begin{aligned}\frac{dP_K^T}{dt} = &\frac{\tilde{f}P_K^L}{V} - d_{PK}P_K^T - p_{PK/x}\phi(y^T)kx^TP_K^T \\ &+ 2^{n_{PK}}p_{PK/x}\phi(y^T(t-n_{PK}\tau))kx^T(t-n_{PK}\tau)P_K^T(t-n_{PK}\tau) \\ &- r_rP_K^T + k\mathcal{J}(p_{TC/B}\phi(y^T), B_{ij}^T)Q_K^T \\ &- p_{TC/R}kP_R^TP_K^T + r_uS_K^T.\end{aligned} \tag{3.15}$$

The first two terms in (3.14) and (3.15) account for the flow of primed T cells out of the lymph node and the natural death rate. The third term of (3.14) accounts for the proliferation of pre-primed CD8+ T cells and corresponds to the second term of (3.11) for pre-primed CD8+ cells. The exponent n_{OK} is the average number of divisions taken by pre-primed CD8+ T cells upon stimulation. As before, the time delay τ is the average amount of time it takes to complete one cell division cycle.

The fourth and fifth terms of (3.14) account for the stimulation and proliferation of primed CD8+ cells upon interacting with a positive growth signal. The coefficient $p_{PK/x}$ is the rate at which a primed CD8+ T cell reacts to a positive growth signal. The third and fourth terms of Eq. (3.15) serve the same function for primed CD8+ cells in the tissue.

The fourth to last terms of (3.14) and (3.15) account for primed CD8+ cells entering the non-proliferating effector state at rate r_r. The third to last terms of both equations are the rates that non-proliferating effectors are restimulated into the primed and proliferating state by interaction with

mature APCs. See Fig. 4 for a diagram of CD8+ cells and Fig. 5 for a diagram of the non-proliferating effector CD8+ state.

The final two terms of each equation account for regulatory mechanism #2 in Fig. 8. As in (3.6) and (3.7), the coefficient $p_{\mathrm{TC/R}}$ is the probability that interactions between a regulatory cell and a primed T cell causes the T cell to become suppressed. Just like $p_{\mathrm{TC/R}}$, this coefficient is also a scalar. The coefficient r_u is the proportional rate that suppressed CD8+ cells, S_K^L and S_K^T, become unsuppressed.

The equations for non-proliferating effector CD8+ cells are

$$\frac{\mathrm{d}Q_K^L}{\mathrm{d}t} = -\tilde{f}Q_K^L - d_{PK}Q_K^L + r_r P_K^L - k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^L), B_{ij}^L)Q_K^L, \tag{3.16}$$

$$\frac{\mathrm{d}Q_K^T}{\mathrm{d}t} = \frac{\tilde{f}Q_K^L}{V} - d_{PK}Q_K^T + r_r P_K^T - k\mathscr{J}(p_{\mathrm{TC/B}}\phi(y^T), B_{ij}^T)Q_K^T. \tag{3.17}$$

The first two terms of (3.16) and (3.17) account for the flow of T cells out of the lymph node and natural death. We assume that non-proliferating effectors die at the same rate d_{PK} as proliferating effectors. The last two terms of both equations account for the transition of primed effectors to the non-proliferating state and vice versa. They are the negatives of the last two terms of (3.14) and (3.15) for primed cells.

3.5. Suppressed CD8+ T cells

The equations for the concentrations of suppressed CD8+ cells S_K^L and S_K^T are

$$\frac{\mathrm{d}S_K^L}{\mathrm{d}t} = -\tilde{f}S_K^L - d_{SK}S_K^L + p_{\mathrm{TC/R}}kP_R^L P_K^L - r_u S_K^L, \tag{3.18}$$

$$\frac{\mathrm{d}S_K^T}{\mathrm{d}t} = \frac{\tilde{f}S_K^L}{V} - d_{SK}S_K^T + p_{\mathrm{TC/R}}kP_R^T P_K^T - r_u S_K^T. \tag{3.19}$$

The first two terms of Eqs. (3.18) and (3.19) account for the flow of suppressed CD8+ cells out of the lymph node and their natural death rate d_{SK}. The final two terms of both equations are the negatives of the final two terms in (3.14) and (3.15). They correspond to the suppression of CD8+ cells by regulatory cells and the natural rate of return to effector activity. See regulatory mechanism #2 in Fig. 8.

3.6. Regulatory cells

The equations for the naturally-occurring regulatory T cell populations are

$$\frac{\mathrm{d}N_R^L}{\mathrm{d}t} = s_R - d_{NH}N_R^L - k\mathscr{J}(p_{\mathrm{R/B}}, B_{ij}^L)N_R^L, \tag{3.20}$$

$$\begin{aligned}\frac{\mathrm{d}P_R^L}{\mathrm{d}t} = {} & -\tilde{f}P_R^L - d_{PH}P_R^L + 2^{n_{NH}}k\mathscr{J}(p_{\mathrm{R/B}}, \\ & B_{ij}^L(t-n_{NH}\tau))N_R^L(t-n_{NH}\tau) \\ & - k\mathscr{J}(p_{\mathrm{R/B}}, B_{ij}^L)P_R^L + 2^{n_{PH}}k\mathscr{J}(p_{\mathrm{R/B}}, \\ & B_{ij}^L(t-n_{PH}\tau))P_R^L(t-n_{PH}\tau),\end{aligned} \tag{3.21}$$

$$\begin{aligned}\frac{\mathrm{d}P_R^T}{\mathrm{d}t} = {} & \frac{\tilde{f}P_R^L}{V} - d_{PH}P_R^T - k\mathscr{J}(p_{\mathrm{R/B}}, B_{ij}^T)P_R^T \\ & + 2^{n_{PH}}k\mathscr{J}(p_{\mathrm{R/B}}, B_{ij}^T(t-n_{PH}\tau))P_R^T(t-n_{PH}\tau),\end{aligned} \tag{3.22}$$

where N_R^L is the concentration of naïve regulatory cells in the lymph node, and P_R^L and P_R^T are the concentrations of primed regulatory cells in the lymph and tissue. These equations are similar to Eqs. (3.4), (3.6), and (3.7) for CD4+ T cells. Here, the matrix $p_{\mathrm{R/B}}$ represents the probabilities that naïve and primed regulatory T cells react to mature APCs. We also assume that naturally-occurring regulatory cells behave in the same way as non-regulatory CD4+ T cell, so we use the same death rates d_{NH} and d_{PH} and the same proliferation rates n_{NH} and n_{PH}. The constant term s_R in equation (3.20) is the constant supply rate of naïve regulatory cells into the lymph node. See Fig. 7.

3.7. Immature (and tolerogenic) APCs

The primary characteristic of tolerogenic APCs that we would like to capture is that they render naïve CD4+ and CD8+ T cells anergic or unresponsive. In this work, due to lack of specific information, we assume the behavior of tolerogenic APCs is the same as that of the immature APCs. Hence, we group immature and tolerogenic APCs into the same population.

The populations of immature APCs are denoted by A_{00}, A_{10}, A_{01}, and A_{11}. The subscripts 00, 10, 01, and 11 correspond to APCs that are presenting neither antigen 1 nor 2, only antigen 1, only antigen 2, and both antigens 1 and 2, respectively. Antigen i is the antigen presented by population T_i.

The equations for immature APC populations A_{00}^L and A_{00}^T that are not presenting either antigen are

$$\begin{aligned}\frac{\mathrm{d}A_{00}^L}{\mathrm{d}t} = {} & s_A - \tilde{f}A_{00}^L - d_A A_{00}^L + \delta_A(A_{10}^L + A_{01}^L) \\ & - p_{\mathrm{A}/\alpha}k(\alpha_1^L + \alpha_2^L)A_{00}^L - r_s A_{00}^L,\end{aligned} \tag{3.23}$$

$$\begin{aligned}\frac{\mathrm{d}A_{00}^T}{\mathrm{d}t} = {} & \frac{\tilde{f}A_{00}^L}{V} - d_A A_{00}^T + \delta_A(A_{10}^T + A_{01}^T) \\ & - p_{\mathrm{A}/\alpha}k(\alpha_1^T + \alpha_2^T)A_{00}^T - r_s A_{00}^T.\end{aligned} \tag{3.24}$$

The term s_A denotes the constant supply of immature APCs to the lymph node. We assume that APCs first enter the lymph node in an immature state and do not present either of the two relevant antigens. The coefficient $\tilde{f}$ is the rate that immature APCs flow out of the lymph node to the tissue. We assume that the flow is unidirectional and that

immature APCs flow out of the lymph node. As discussed before, the flow terms in the first and second equations have opposite signs and differ by the volume ratio V.

The coefficient d_A denotes the death rate of APCs, and the coefficient δ_A denotes the turnover rate of antigen on the surfaces of immature APCs. That is to say, the immature APC populations A_{10} and A_{01} that are presenting either antigen 1 or 2 return to a state of not presenting either antigen at rates $\delta_A A_{10}$ and $\delta_A A_{01}$, respectively.

The coefficient $p_{A/\alpha}$ is the probability with which APCs incorporate and present the encountered antigen. Hence, the terms $-p_{A/\alpha}k(\alpha_1+\alpha_2)A_{00}$ in both equations is the rate at which immature APCs are promoted to either the A_{10} or A_{01} state.

The final terms of (3.23) and (3.24), are the rate at which immature APCs mature due to an inflammatory signal. During infection, we expect the rate r_s to be high, whereas in a non-infectious state, we expect r_s to be low or almost zero.

Let

$$A_1 = \begin{bmatrix} A_{10} \\ A_{01} \end{bmatrix}.$$

Then the equations for immature APCs that are presenting either antigen 1 or 2 exclusively are

$$\begin{aligned} \frac{dA_1^L}{dt} = & -\tilde{f}A_1^L - d_A A_1^L - \delta_A A_1^L + \frac{1}{2}\delta_A A_{11}^L + p_{A/\alpha}k\alpha^L A_{00}^L \\ & - p_{A/\alpha}k\begin{bmatrix} \alpha_2^L \\ \alpha_1^L \end{bmatrix} A_1^L - r_s A_1^L - k(p_{A/PH}\phi(y^L)P_H^L)A_1^L \\ & + k(p_{B/R}P_R^L)B_1^L, \end{aligned} \tag{3.25}$$

$$\begin{aligned} \frac{dA_1^T}{dt} = & \frac{\tilde{f}A_1^L}{V} - d_A A_1^T - \delta_A A^T i1 + \frac{1}{2}\delta_A A_{11}^T + p_{A/\alpha}k\alpha^T A_{00}^T \\ & - p_{A/\alpha}k\begin{bmatrix} \alpha_2^T \\ \alpha_1^T \end{bmatrix} A_1^T - r_s A_1^T - k(p_{A/PH}\phi(y^T)P_H^T)A_1^T \\ & + k(p_{B/R}P_R^T)B_1^T. \end{aligned} \tag{3.26}$$

The first two terms of each equation are the flow rate and death rate terms as before.

Immature APCs presenting antigen 1 or 2 stop presenting that antigen at rate δ_A, which corresponds to the turnover of surface molecules of immature APCs. Hence, the third terms of (3.25) and (3.26) are the rates that APCs A_{10} and A_{01} return to state A_{00} where they are presenting neither antigen 1 nor 2. Similarly, APCs A_{11} that are presenting both antigens lose one of the antigens at rate $\delta_A A_{11}$. We assume that the probabilities of losing antigen 1 or 2 are equal. Hence, the fourth term of (3.25) and (3.26) is the rate that APCs A_{11} drop to either state A_{10} or A_{01} and are half the rate $\delta_A A_{11}$.

Meanwhile, APCs A_{00} that are not presenting any antigen continue to pick up circulating antigen and present them at rate $p_{A/\alpha}k\alpha^T A_{00}^T$, where $p_{A/\alpha}$ is the probability that immature APCs present encountered antigen. Recall that α is a 2×1 vector representing both antigen populations. Hence, the fifth terms of both equations are the rates that APCs A_{00} start to present antigen 1 or 2. Similarly, the sixth terms are the rates that APCs presenting antigen 1 pick-up and present antigen 2 and vice versa. The sixth terms are the rates that APCs A_{10} and A_{01} are promoted to state A_{11} where they are presenting both antigens.

The penultimate terms are the rates these APCs mature due to an inflammatory signal, and r_s is the proportional rate of simulation. The eighth terms are the rates at which these APCs are stimulated to mature due to interactions with primed CD4+ T cells. The 2×2 matrix $p_{A/PH}$ is the probability that interactions between primed CD4+ T cells and immature APCs results in a successful stimulation of the APCs. Again, these terms represent stimulatory interactions between APCs and T cells, so they are adjusted by the decay function ϕ due to negative signaling.

The final terms account for regulatory mechanism #3 in Fig. 8. We assume interactions between regulatory cells and APCs are antigen-specific and mutual (i.e., either both cells react or both cells remain unaffected). Thus the 2×2 matrix $p_{B/R}$ is the same as the one used in (3.21) and (3.22). It denotes the probabilities that interactions between mature APCs and regulatory cells cause the APCs to become tolerogenic.

The equations for immature APCs that are presenting both antigens 1 and 2 are

$$\begin{aligned} \frac{dA_{11}^L}{dt} = & -\tilde{f}A_{11}^L - d_A A_{11}^L - \delta_A A_{11}^L + p_{A/\alpha}k\begin{bmatrix} \alpha_2^L & \alpha_1^L \end{bmatrix} A_1^L \\ & - r_s A_{11}^L - k\mathcal{K}(p_{A/PH}\phi(y^L), P_H^L)A_{11}^L \\ & + k\mathcal{K}(p_{B/R}, P_R^L)B_{11}^L, \end{aligned} \tag{3.27}$$

$$\begin{aligned} \frac{dA_{11}^T}{dt} = & \frac{\tilde{f}A_{11}^L}{V} - d_A A_{11}^T - \delta_A A^T i11 + p_{A/\alpha}k\begin{bmatrix} \alpha_2^T & \alpha_1^T \end{bmatrix} A_1^T \\ & - r_s A_{11}^T - k\mathcal{K}(p_{A/PH}\phi(y^T), P_H^T)A_{11}^T \\ & + k\mathcal{K}(p_{B/R}, P_R^T)B_{11}^T. \end{aligned} \tag{3.28}$$

The first three terms of (3.27) and (3.28) account for the flow out of the lymph node, natural death rate, and surface antigen turnover rate respectively. The fourth terms are the row sums of the sixth terms of (3.25) and (3.26). They correspond to the promotion of APCs A_{I10} and A_{I01} to the state A_{I11}. The fifth terms are the rates these APCs mature due to inflammation.

The penultimate terms are the rate these APCs are stimulated to mature due to interactions with primed CD4+ T cells. The function $\mathcal{K}$ maps to a scalar and is defined by

$$\mathcal{K}(p, P_H) = \begin{bmatrix} 1-(1-p_{11})(1-p_{21}) \\ 1-(1-p_{12})(1-p_{22}) \end{bmatrix}^T P_H, \tag{3.29}$$

where p is a 2×2 matrix and P_H is a 2×1 vector containing both CD4+ populations. The function $\mathcal{K}$ maps to a scalar that denotes the rate that an APC presenting both antigens reacts to the ensemble of both kinds of CD4+ cells. As usual, these terms are adjusted due to

negative signaling. The final terms are the rates that mature APCs are tolerized by interactions with regulatory T cells.

3.8. Mature APCs

The equations for the mature APC population B_{00}^L and B_{00}^T that are not presenting either antigen are

$$\frac{dB_{00}^L}{dt} = fB_{00}^T - d_A B_{00}^L + \delta_{Am}(B_{10}^L + B_{10}^L) + r_s A_{00}^L, \tag{3.30}$$

$$\frac{dB_{00}^T}{dt} = -\frac{fB_{00}^T}{V} - d_A B_{00}^T + \delta_{Am}(B_{10}^T + B_{01}^T) + r_s A_{00}^T. \tag{3.31}$$

The first terms of (3.30) and (3.31) represent the migration of mature APCs into the lymph node. We assume that the flow is unidirectional and that mature APCs flow into the lymph node. The second terms represent the natural death of APCs at rate d_A. The third terms are analogous to the fourth and third terms of (3.23) and (3.24) for immature APCs and represent the rate that mature APCs return to state B_{00} due to surface molecule turnover. The final terms are the rates that immature APCs are promoted to the mature state due to inflammation. These terms are the negatives of the final terms of (3.23) and (3.24) for immature APCs.

Let $B_1 = [B_{10} B_{01}]^T$. Then the equations for mature APC populations that are presenting either antigen 1 or 2 exclusively are

$$\begin{aligned}\frac{dB_1^L}{dt} &= fB_1^T - d_A B_1^L - \delta_{Am} B_1^L + \frac{1}{2}\delta_{Am} B_{11}^L \\ &\quad + r_s A_1^L + k(p_{\mathrm{A/PH}}\phi(y^L)P_H^L)A_1^L \\ &\quad - k(p_{\mathrm{B/R}}P_R^L)B_1^L,\end{aligned} \tag{3.32}$$

$$\begin{aligned}\frac{dB_1^T}{dt} &= -\frac{fB_1^T}{V} - d_A B_1^T - \delta_{Am} B_1^T + \frac{1}{2}\delta_{Am} B_{11}^T \\ &\quad + r_s A_1^T + k(p_{\mathrm{A/PH}}\phi(y^T)P_H^T)A_1^T \\ &\quad - k(p_{\mathrm{B/R}}P_R^T)B_1^T.\end{aligned} \tag{3.33}$$

The first four terms of (3.32) and (3.33) are analogous to the first four terms of (3.25) and (3.26). The last three terms of (3.32) and (3.33) are negatives of the last three terms of (3.25) and (3.26), because they correspond to transitions from the immature to mature state and vice versa.

The equations for mature APCs that are presenting both antigens 1 and 2 are

$$\begin{aligned}\frac{dB_{11}^L}{dt} &= fB_{11}^T - d_A B_{11}^L - \delta_{Am} B_{11}^L \\ &\quad + r_s A_{11}^L + k\mathcal{K}(p_{\mathrm{A/PH}}\phi(y^L), P_H^L)A_{11}^L \\ &\quad - k\mathcal{K}(p_{\mathrm{B/R}}, P_R^L)B_{11}^L,\end{aligned} \tag{3.34}$$

$$\begin{aligned}\frac{dB_{11}^T}{dt} &= -\frac{fB_{11}^T}{V} - d_A B_{11}^T - \delta_{Am} B_{11}^T \\ &\quad + r_s A_{11}^T + k\mathcal{K}(p_{\mathrm{A/PH}}\phi(y^T), P_H^T)A_{11}^T \\ &\quad - k\mathcal{K}(p_{\mathrm{B/R}}, P_R^T)B_{11}^T.\end{aligned} \tag{3.35}$$

The first three terms of (3.34) and (3.35) are analogous to the first three terms of (3.27) and (3.28). The last three terms of (3.34) and (3.35) are negatives of the last three terms of (3.27) and (3.28), because they correspond to transitions from the immature to the mature state and vice versa.

3.9. Positive signals

The equations for the concentrations x^L and x^T of the positive growth signal are

$$\begin{aligned}\frac{dx^L}{dt} &= s_{x,1}\phi(y^L)(P_{H,1}^L + P_{H,2}^L) + s_{x,2}\phi \\ &\quad \times(y^L)(O_{K,1}^L + O_{K,2}^L) + fx^T - d_x x^L \\ &\quad - k(P_{K,1}^L + P_{K,2}^L + O_{K,1}^L + O_{K,2}^L)x^L - kP_R^L x^L,\end{aligned} \tag{3.36}$$

$$\begin{aligned}\frac{dx^T}{dt} &= s_{x,1}\phi(y^T)(P_{H,1}^T + P_{H,2}^T) - \frac{fx^T}{V} - d_x x^T \\ &\quad - k(P_{K,1}^T + P_{K,2}^T)x^T - kP_R^T x^T.\end{aligned} \tag{3.37}$$

The first two terms in (3.36) are the rates at which the positive growth signal is secreted by primed CD4+ and pre-primed CD8+ T cells in the lymph node. Here, $s_{x,1}$ and $s_{x,2}$ are the proportional rates of secretion. The first term in (3.37) is the rate at which the positive growth signal is secreted by primed CD4+ T cells in the tissue. We assume that the secretion rates of positive signal are diminished by negative signaling, so these terms are adjusted by the decay function ϕ.

The fourth to last terms in both equations represent the drainage from the tissue to the lymph node. The third to last terms are the natural decay rates of growth signal that is not consumed. The penultimate terms are the rates in which a positive growth signal is consumed by proliferating CD8+ cells (primed and pre-primed). For simplicity, we assume that T cells take up any positive growth signal they encounter. Hence, there is no probability term associated with the consumption of positive signals. The final terms in both equations account for regulatory mechanism #4 in Fig. 8. They represent the rate at which positive signal is taken up by circulating regulatory cells.

3.10. Negative signals

Negative signals are due to regulatory mechanism #5 in Fig. 8. The equations for the concentrations of the negative signal, y^L and y^T, are

$$\begin{aligned}\frac{dy^L}{dt} &= s_y P_R^L + fy^T - d_x y^L - k(P_{H,1}^L + P_{H,2}^L \\ &\quad + P_{K,1}^L + P_{K,2}^L + O_{K,1}^L + O_{K,2}^L)y^L,\end{aligned} \tag{3.38}$$

$$\frac{dy^T}{dt} = s_y P_R^T - \frac{fy^T}{V} - d_x y^T - k(P_{H,1}^L + P_{H,2}^L + P_{K,1}^L + P_{K,2}^L)y^T. \tag{3.39}$$

The coefficient s_y is the rate at which regulatory cells secrete a negative signal and d_x is the natural decay rate of circulating growth signal. The third terms account for the drainage of signal into the lymph node at rate f. We assume that signal drains passively into the lymph node at the same rate as antigen. The final terms in both equations correspond to the rate of consumption of a negative signal by CD4+ cells and primed and pre-primed CD8+ cells.

4. Parameter estimates

Due to the difficulty of obtaining immunological data from humans, many quantitative in vivo experiments involve mice rather than humans. In our parameter estimates, we use both human and mouse data and assume, wherever possible, that the mouse data give reasonable estimates for human parameters. In our tables, we indicate which references correspond to human data (with a superscript $(\cdot)^H$) and which correspond to mouse data (with a superscript $(\cdot)^M$).

4.1. Cell concentrations in the lymph node

Catron et al. (2004, p. 341b), consider a hypothetical, spherical, skin-draining lymph node of diameter 2 mm (or radius 1 mm). Such a lymph node would have volume $(4\pi/3)(0.1\,\text{cm})^3 = 0.0042\,\text{mL} = 4.2\,\mu\text{L}$.

Furthermore, Catron et al. estimate that a lymph node of this size would contain 4×10^6 total lymphocytes of which 1.2×10^6 are CD4+ T cells. This leads to an estimate of the CD4+ T cell concentration of $1.2 \times 10^3\,\text{k}/4.2\,\mu\text{L} = 286\,\text{k}/\mu\text{L}$, or about $300\,\text{k}/\mu\text{L}$, in the lymph node.

In their model, they simulate the a slice of about $\frac{1}{500}$ the total volume of the lymph node. They estimate the slice to contain about 2400 CD4+ T cells, 1600 CD8+ T cells, and 100 DCs (Catron et al., 2004, p. 342b). Based on these proportions, we obtain a CD8+ T cell concentration of $300\,\text{k}/\mu\text{L} \times \frac{1600}{2400} = 200\,\text{k}/\mu\text{L}$ and a DC concentration of $300\,\text{k}/\mu\text{L} \times \frac{100}{2400} = 12\,\text{k}/\mu\text{L}$ in the lymph node. Since DCs are the primary APCs that stimulate T cells, we assume that our estimates for DCs are good estimates for the APC concentration in our model. The estimates for the cell concentrations in the lymph node are listed in Table 1.

4.2. Cell concentrations in the tissue

The steady state CD4+ T cell concentration in blood is estimated to be about $1\,\text{k}/\mu\text{L}$ (Mohri et al., 2001; Snedecor, 2003), and the steady state CD8+ T cell concentration in the blood is estimated to be about $0.6\,\text{k}/\mu\text{L}$ (Mohri et al., 2001). Note that the ratio of CD4+ and CD8+ T cell concentrations in the blood from this estimate is about 3:2, which is the same as the ratio in the lymph node estimated in Catron et al. (2004). These results are mutually corroborative and also imply that the flow rates of CD4+ and CD8+ T cells between the tissue and lymph node are similar, if not identical.

Table 1
Steady-state cell concentrations

Parameters	Estimate (k/μL)	References
Cell concentrations in the lymph node		
CD4+ T cell concentration	300	(Catron et al., 2004)^M
CD8+ T cell concentration	200	(Catron et al., 2004)^M
APC concentration	12	(Catron et al., 2004)^M
Cell concentrations in the tissue		
CD4+ T cell concentration	1	(Lee ; Snedecor, 2003)^H
CD8+ T cell concentration	0.6	(Lee)^H
APC concentration	0.04	(Catron et al., 2004)^M, assumption

If we assume that the proportion of APCs inside and outside the lymph node is that of the T lymphocytes, we estimate that the concentration of APCs in the tissue is $\frac{12}{300} = 0.04\,\text{k}/\mu\text{L}$. The estimates for the cell concentrations in the tissue are listed in Table 1.

4.3. Flow rates between lymph node and tissue

Recall from the equations in Section 3 that f and $\tilde{f}$ denote the flow rates into and out of the lymph node, respectively. In addition, the change in the concentration of some population X in the LN due to inflow is $f \times X^T$, while the change in the LN due to outflow is $-\tilde{f}X^L$. Similarly, the change in the concentration in the tissue due to inflow is $\tilde{f}X^L/V$, while the change due to the outflow is $-fX^T/V$. Here, $V = (\text{volume of tissue})/(\text{volume of LN})$.

Haase (1999) and Snedecor (2003) estimate that lymphocytes spend $p = 98\%$ of their time in the lymph node and 2% of their time in the periphery, or tissue. From this information, the ratio of lymphocyte concentrations between the lymph node and tissue is $98\%/(2\%/V) = 49V$. From Table 1, we also estimate this ratio to be 300 to 1. This implies that $V = 300/49 \sim 6$.

Expressing populations in terms of cells instead of concentrations, Snedecor estimates the flow rate of T cells from blood to the lymph to be 0.05/day (Snedecor, 2003). Assuming that $V = 6$, this estimate corresponds to a flow rate of $f_{(\text{T cell})} = 0.05 \times V = 0.3/\text{day}$, when populations are expressed in terms of concentrations. Applying the ratio 98% to 2%, we obtain $\tilde{f} = (f_{(\text{T cell})}/V)/49 \times 10^{-3}$. However, we note that the model in (Snedecor, 2003) considers only CD4+ cells as one population rather than dividing them into naïve and primed states. In our model, we assume that naïve CD4+ cells remain in the lymphoid tissue, while primed CD4+ cells tend to emigrate to the periphery. Hence, we assume that the flow of primed CD4+ cells is unidirectional out of the lymph node and that backflow is negligible. Hence, we assume the outflow

rate of primed CD4+ cells is $\tilde{f} = 10^{-3}$ as estimated and the inflow rate of primed CD4+ cells is 0. Furthermore, naïve CD4+ cells remain in the lymph node and account for most of its CD4+ population in the absence of an immune response. We assume these flow rates also apply to CD8+ cells.

Regarding APCs, Catron et al. (2004) estimate that after a DC matures, it takes about 18 h for it to detach and appear in the lymph node. Hence, assuming a half life of 18 h = 0.75 days, we estimate the flow rate f of mature APCs into the lymph node to be $(\log(2)/0.75)V \sim 5.5$. Under steady-state conditions, we assume that most APCs are immature, and they flow from the lymph node to the tissue compartment at the same rates as T cells. Hence, the flow rate of immature APCs is also $\tilde{f} = 10^{-3}$/day.

Antigens drain from infected tissue into the lymph node, where they are picked up by resident APCs. This rate of drainage exceeds the rate of immigration of APCs from the tissue. In the animated simulations of Catron et al. (2004), draining antigen appears in the lymph node about 3 h after injection. However, the draining antigen does not immediately appear at a high concentration, and it is only after the arrival of a mature antigen-bearing APC about 18 h later that T cells begin to get stimulated (Catron et al., 2004). Hence, circulating antigen may appear quickly in the lymph node due to its high concentration in the tissue, but its proportional drainage rate may be low. In the absence of unequivocal data regarding the rate of antigen drainage, we assume that this rate is the same as the inflow rate of mature APCs, i.e., $f = 5.5$/day. See Table 2 for a complete list of the flow rates estimates.

4.4. Supply and death rates

We assume that naïve immune cells are supplied by stem cells into the lymph node and express concentrations in terms of the concentration in the lymph node. From Table 1, we know that the lymph node concentration of total CD4+ cells is 300 k/μL and that the tissue concentration is 1 k/μL. Hence, the total lymph node concentration would be $300 + 10 \times 1 = 310$ k/μL, if all the cells were packed in the lymph node. The contribution of cells from the tissue is not very significant. In fact, this is true for all three cell concentrations, because most of the cells are in the lymph node at any given time. Hence, we can use the lymph node concentration as a good indicator of the total amount of cells. Furthermore, we take the following estimates to apply primarily to naïve T cells and assume that most T cells are naïve in the steady state.

From (Mohri et al., 2001), we have approximately a 3% supply rate of CD4+ and CD8+ T cells per day at steady state. Since these steady state populations are at equilibrium, it follows that the net death rate is also around 3% per day. Proliferation is less significant at about 0.3–0.4% per day. This gives a death rate of $-\log(100\% - 3\%) = 0.03$/day for T cells, so the supply rate for CD4+ T cells is $300\,\text{k}/\mu\text{L} \times 3\%/\text{day} = 9\,\text{k}/\mu\text{L}\,\text{day}^{-1}$, and the supply rate for CD8+ T cells is $200\,\text{k}/\mu\text{L} \times 3\%/\text{day} = 6\,\text{k}/\mu\text{L}\,\text{day}^{-1}$. Since cells supplied into the system are presumably, naïve, we assume these turnover estimates apply to naïve T cells.

Since we assumed that regulatory cells die at the same rate as non-regulatory CD4+ cells, and they represent about 3–15% of CD4+ cells in humans (Janeway et al., 2005; Walker et al., 2005), we estimate that about 5% of CD4+ cells are regulatory and are supplied at a rate of $5\% \times 9 = 0.45\,\text{k}/\mu\text{L}\,\text{day}^{-1}$ for regulatory CD4+ cells and $95\% \times 9 = 8.55\,\text{k}/\mu\text{L}\,\text{day}^{-1}$ for naïve non-regulatory CD4+ cells.

Table 1 lists estimated concentrations for all CD4+ and CD8+ cells, but we are interested in the subset of T cells that are reactive to the antigen presented by a certain cell. From De Conde et al. (2005), we have the estimate that about $\frac{1}{5000}$ TCR specificities are tumor reactive, and from Catron et al. (2004), we have the estimate that about $\frac{1}{5000}$ naïve CD4+ T cells are LCMV specific. Thus, we use $\frac{1}{5000}$ as our estimate for the frequency ω of antigen-specific naïve T cells.

We do not have explicit references for the supply and death rates of APCs so we assume that they are similar to those of naïve T cells, and hence the supply rate for APCs cells is $12\,\text{k}/\mu\text{L} \times 3\%/\text{day} = 0.36\,\text{k}/\mu\text{L}\,\text{day}^{-1}$. Note that APCs are not antigen-specific in the same way that T cells are. We also assume that the death rate of mature and immature APCs is the same (Table 3).

4.5. Effector death and proliferation rates

Primed CD8+ T cells die much faster than naïve T cells. Using unpublished data from Lee, we estimate the halflife of effector CD8+ T cells to be about 24 h. Hence, we have an effector death rate of $\log(2)/(1\text{ day}) = 0.69$/day. In addition, Duvall and Perry give the estimate that effector T cells have a half-life of three days (Duvall and Perry, 1968), which yields a death rate of $\log(2)/(3\text{ day}) = 0.23$/day. Hence, we estimate that the death rate of primed CD8+ T cells is the range of 0.2–0.7/day. We also assume that the death rate of primed CD4+ T cells falls in this range.

By applying mathematical models, it is estimated in De Boer et al. (2003) that primed CD4+ T cells initially die with a halflife of 3 days and later slow down to a halflife of

Table 2
Flow rates between the lymph node and the tissue

Parameters	Description	Estimate	References
V	Tissue to lymph volume ratio	6	follows from (Snedecor, 2003)^H and Table 1
f	Mature APC and antigen inflow rate	5.5/day	(Catron et al., 2004)^M
$\tilde{f}$	T cell and immature APC outflow rate	10^{-3}/day	(Snedecor, 2003)^H

Table 3
Supply and death rates of naïve T cells and DCs

Parameters	Description	Estimate	References
ω	Freq. of antigen-reactive T cells	$\frac{1}{5000}$	(Catron et al., 2004)^M, (De Conde et al., 2005)^H
d_{NH}	Naïve CD4+ death rate	0.03/day	(Lee)^H
d_{regn}	Naïve T_{reg} death rate	0.03/day	Assume same as d_{NH}
d_{NK}	Naïve CD8+ death rate	0.03/day	(Lee)^H
d_A	APC death rate	0.03/day	Assume same as d_{NH}, d_{NK}
s_H	CD4+ supply rate	$8.55\,\mathrm{k/\mu L\,day^{-1}}$	(Lee)^H
s_R	T_{reg} supply rate	$0.45\,\mathrm{k/\mu L\,day^{-1}}$	Based on (Lee ; Janeway et al., 2005)^H, (Sakaguchi et al., 1995)^M
s_K	CD8+ supply rate	$6\,\mathrm{k/\mu L\,day^{-1}}$	(Lee)^H
s_A	APCsupply rate	$0.36\,\mathrm{k/\mu L\,day^{-1}}$	Calculated from total APC population and death rate

Table 4
Division and death rates of primed T cells

Parameters	Description	Estimate (Range)	References
d_{PH}	Primed CD4+ death rate	0.2/day (0.2–0.7)	(Duvall and Perry, 1968; Lee)^H, (De Boer et al., 2003)^M
d_{PK}	Primed CD8+ death rate	0.4/day (0.2–0.7)	(Duvall and Perry, 1968; Lee)^H, (De Boer et al., 2003)^M
n_{NH}	CD4+ divisions upon activation	1	Assumption
n_{PH}	CD4+ divisions upon further stimulation	1	Assumption
n_{OK}	CD8+ divisions upon activation	8 (8–10)	(Antia et al., 2003; Janeway et al., 2005)^H
n_{PK}	CD8+ divisions upon further stimulation	2	Estimation
τ	Duration of T cell division	0.6 day (0.25–1)	(Janeway et al., 2005; Lee; Luzyanina et al., 2004)^H

35 days. These numbers yield an initial death rate of 0.23/day and an eventual death rate of 0.02/day. In this model, we will assume a constant death rate for CD4+ cells of 0.2/day. In addition, (De Boer et al., 2003) estimates that primed CD8+ T cells die with a halflife of 1.7 days, which yields a death rate of about 0.4/day.

When naïve CD8+ T cells are primed for the first time, they go through several cycles of division. An analysis of experimental data by Antia et al. (2003) showed that stimulation of naïve CD8+ cells result in up to eight divisions in vitro (Antia et al., 2003; Wong and Pamer, 2001). In addition, Janeway et al. (2005, p. 19) estimates that the proliferation of primed CD8+ cells leads to about 10^3 daughter cells, which implies about 10 divisions. Hence, we estimate that naïve CD8+ T cells go through 8–10 divisions upon initial stimulation. Primed CD8+ T cells continue to divide as long as they receive a positive growth signal. We estimate they divide twice every time they receive a sufficient stimulus.

In general, CD4+ T cells do not divide as much as CD8+ T cells. Furthermore, CD4+ proliferation seems to stop soon after the antigen stimulus is removed, whereas CD8+ proliferation continues (Mercado et al., 2000). Hence, we assume that CD4+ T cells divide once upon activation and once more upon each additional stimulation by DCs.

Luzyanina et al. (2004), estimate that T cell division takes between 0.4–2 days, and their best fit estimate is 0.6 days. In unpublished data, Lee estimates that T cell division take about 24 h (Lee). Also, Janeway et al. (2005) estimates that primed T cells divide 2–4 times per day, which corresponds to a duration of 0.25–0.5 days. Combining these sources, we estimate that T cell division takes between 0.25 and 1 day, and we favor the estimate of 0.6 days from (Luzyanina et al., 2004), see Table 4.

4.6. Cytokines, antigens, and collision rates

Upon activation CD4+ T cells secrete cytokines, mainly the positive growth signal IL-2, which stimulate primed CD8+ T cells to divide. IL-2 also stimulates CD4+ T cells to further division, but since this is an autocrine loop we assume CD4+ cells are self-sufficient to divide and do not need to interact with circulating positive growth signals to divide. We know that activation of T cells increases their IL-2 production by up to 100-fold (Janeway et al., 2005, Sections 8–10). Furthermore, (Kasaian et al., 1991) presents in vitro data that mice that are >90% depleted of CD4+ cells have a 90–99 % drop in IL-2 production. From this information, we approximate that primed CD4+ cells produce 10 times as much positive signal as primed CD8+ cells and estimate that the rates of secretion of positive signal by T cells are $s_{x,1} = 100\,\mathrm{k/\mu L\,cell^{-1}\,day^{-1}}$ for CD4+ cells and $s_{x,2} = 10\,\mathrm{k/\mu L\,cell^{-1}\,day^{-1}}$ for pre-primed CD8+ cells.

We also estimate that one primed or pre-primed CD8+ T cell interacting with one unit of positive signal over one day has a 50% chance of dividing. Hence, the corresponding rate coefficient, $p_{\mathrm{PK/x}}$, is 0.5.

From in vitro multiplication of CD8+ cells, it is known that IL-2 breaks down very rapidly, so we model that

unconsumed signals decay with a half life of 3 h, giving a decay rate of $d_x = 5.6/\text{day}$.

For our model, we assume that on average virus-infected and normal self cells produce a certain amount of antigen upon death. Very little is known about the rate that antigen is produced or how effectively it is incorporated by APCs. The rate of antigen production may vary from cell to cell, but we estimate that the antigen secretion rate s_α is the same as the rate of secretion of positive signals by CD4+ cells, i.e., $100\,\text{k}/\mu\text{L}\,\text{cell}^{-1}\,\text{day}^{-1}$. Also, we estimate that one APC interacting with one unit of antigen over one day has a 50% chance of effectively incorporating and presenting that antigen. Hence, the rate coefficient is $p_{A/\alpha} = 0.5$. Finally, we assume that a circulating antigen that is not taken up decays at the same rate as positive and negative signal, giving a decay rate of $d_\alpha = 5.6/\text{day}$.

Catron et al. (2004) estimate that in their simulated lymph node slice, one T cell and one DC will have 0.20 ± 0.06 T cell-DC contacts per hour. Recall from Section 4.1 that their hypothetical lymph node has a volume of $4.2\,\mu\text{L}$ and their slice is $\frac{1}{500}$ the lymph node volume. Hence one T cell in the slice has a concentration of $1\,\text{cell}/(4.2\,\mu\text{L}/500) = 0.5/4.2\,\text{k}/\mu\text{L}$. By the law of mass action, we assume that the collision rate of the cells is of the form

$$k[\text{T cells}][\text{DCs}],$$

where k is the kinetic coefficient. Hence, we have

$$\begin{aligned} k[\text{one T cell}][\text{DC}] &= (0.20\,\text{contacts/h})[\text{APC}] \\ &= (4.8\,\text{contacts/day})\,[\text{DC}], \end{aligned}$$

which implies that $k = (4.8/\text{day}) \times (0.5/4.2\,\text{k}/\mu\text{L}) = 40(\text{k}/\mu\text{L}\,\text{day})^{-1}$.

DCs continually degrade surface molecules, and immature DCs have a higher turnover rate than mature DCs (Wilson et al., 2004). In particular, experiments by Wilson et al. (2004) have shown that after 80 min, immature DCs degraded 70% of their surface MHC II molecules, while mature DCs degraded only 85%. Their results for MHC I molecules are similar. Hence, we obtain surface molecule degradation rates of

$$\begin{aligned} \delta_A &= -\log(100\% - 70\%)/(80\ \text{min}) \\ &= 21.6542/\text{day} \sim 20/\text{day}, \end{aligned}$$

$$\begin{aligned} \delta_{Am} &= -\log(100\% - 15\%)/(80\ \text{min}) \\ &= 2.9230/\text{day} \sim 3/\text{day}. \end{aligned}$$

Also, we assume that APCs immature continuously mature at rate r_s due to circulating inflammatory signal. From non-infectious to infectious states, this rate should increase from being relatively low to relatively high. We assume that these rates are 0.01/day and 1/day, respectively. These rates correspond to $1 - e^{-0.01} = 1\%$ and $1 - e^{-1} = 63\%$ of immature APCs maturing per day. See Table 5 for a summary of these parameters.

Table 5
Secretion rates and kinetic coefficient

Parameters	Description	Estimate	References
k	Kinetic coefficient	$40(\text{k}/\mu\text{L}\,\text{day})^{-1}$	(Catron et al., 2004)^H
s_α	Antigen secretion rate	100/day	Estimation
$s_{x,1}$	Positive growth signal secretion rate by CD4+'s	100/day	Estimation
$s_{x,2}$	Positive growth signal secretion rate by CD8+'s	10/day	Estimation
d_α	Circulating antigen decay rate	0.1/day	Estimation
d_x	Growth signal decay rate	0.1/day	Estimation
$p_{\text{PK}/x}$	Rate of CD8+ cell response to growth signal	0.5	Estimation
$p_{A/\alpha}$	Rate of antigen incorporation by APCs	0.5	Estimation
δ_A	Degradation rate of surface molecules on immature APCs	20/day	(Wilson et al., 2004)^H
δ_{Am}	Degradation rate of surface molecules on mature APCs	3/day	(Wilson et al., 2004)^H
$r_{s,0}$	Rate of maturation of APCs in non-infectious conditions	0.01/day, 1% of APCs per day	Estimation
r_s	Rate of maturation of APCs during infection	1/day, 63% of APCs per day	Estimation

4.7. State transitions for T cells

Recall from Fig. 5 that effector CD8+ cells can transition from a primed and proliferating state to a non-proliferating state at rate r_r. Also recall from Fig. 8 that suppressed T cells return to effector states at rate r_u.

There are certain types of suppression that are permanent as in T cell anergy; however, there is evidence for temporary modes of suppression that can be reversed under the appropriate stimulus. For example in a study of HIV, Trimble and Leiberman show that in an IL-2-rich environment, downregulated CD8+ cells can be restored to viral-specific cytotoxicity in 6–16 h (Trimble and Lieberman, 1998).

Hence, there is evidence of state transitions from dormant or downregulated states, and we estimate that both of these state transitions occur with a halflife of about 1 day, which yields values of $r_r = 0.7/\text{day}$ and r_u/day. See Table 6 for a table that summarizes these parameters.

4.8. Probabilities of interactions

Regarding the probabilities of productive cell-to-cell interactions, we assume that some reactivities are high, such as anti-foreign effector reactivities, while others are low, such as anti-self effector reactivities. In Table 7, we list our estimated ranges for these probability values.

In modeling the effects of negative signals that are secreted by regulatory cells, we assume that several probability and interaction rates decrease exponentially as a function of the concentration of negative signals. The rate of the exponential decrease is c and is estimated as $1(\mathrm{k}/\mu\mathrm{L}\,\mathrm{day})^{-1}$.

4.9. Target-related parameters

We assume that potential target cells, either virus-infected or normal self cells, come from two potential sources: stem cell supply from the body or natural growth and expansion. The first source should only apply to normal self cells or abnormal self cells, such as cancer.

The turnover rate of normal self cells ranges from a half-life of hours as with neutrophils to a half-life of months to years. Hence, we estimate a turnover rate of 1%, i.e. the natural death rate $d_{T,1}$ for normal self cells is 0.01/day, corresponding to a half-life of slightly over two months. We also assume that self cells have a zero growth rate and a supply rate $s_{T,1}$ of 0.1 $(\mathrm{k}/\mu\mathrm{L})$/day, giving an equilibrium normal self population of $10\,\mathrm{k}/\mu\mathrm{L}$. In addition, we assume that the growth rate of foreign target is 0.3/day and its death rate is 0.1/day. See Table 8 for a list of parameters.

4.10. Initial concentrations

To set the initial history, we assume that all populations start at a steady state with no active immune reactions. The latter assumption eliminates all bilinear terms to the model equations. Finding the steady-state solutions involves calculating the fixed point of the remaining linear system. We discuss how we calculate these solutions in Appendix C.

Table 6
T cell transition rates

Parameters	Description	Estimate	References
r_r	Rate primed CD8s enter non-proliferating state	0.7/day	Estimation
r_u	Rate suppressed T cells become unsuppressed	0.7/day	Estimation

Table 8
Target supply, growth, and death rates

Parameters	Description	Estimate	References
s_T	Target cell supply rate	self = $0.1(\mathrm{k}/\mu\mathrm{L})$/day foreign = 0	Varies across target types
g_T	Target cell growth rate	self = 0 foreign = 0.3	
d_T	Target cell death rate	self = 0.01/day foreign = 0.1/day (1/2-life = 7 days)	

Table 7
Interaction probabilities

Parameters	Description	Estimate	References
$p_{\mathrm{PK/T}}$ ($[p_{\mathrm{T/PK}}]^t$)	Prob of cytotoxic CD8+ reaction to target	anti-foreign $\in [0,1]$ anti-self $\in [0,0.1]$	Assumption: anti-foreign is high and anti-self is low
$p_{\mathrm{TC/A}}$	Prob of tolerizing reaction between naïve T cells and immature APCs	anti-foreign $\in [0,0.1]$ anti-self $\in [0,0.1]$	Assumption: both are low
$p_{\mathrm{TC/B}}$	Prob of stimulating interaction between T cells and mature APCs	anti-foreign $\in [0,1]$ anti-self $\in [0,0.1]$	Assumption: anti-foreign is high, and anti-self is low
$p_{\mathrm{A/PH}}$	Prob of stimulating interaction between immature APCs and primed CD4+ cells	$p_{\mathrm{TC/B}}$	Assumption: same as the prob of the stimulation of naïve CD4+ cells by mature APCs
$p_{\mathrm{R/B}}$	Prob of stimulating interaction between regulatory cells and mature APCs	anti-foreign $\in [0,1]$ anti-self $\in [0,1]$	Assumption: anti-foreign may be high or low, and anti-self is high
$p_{\mathrm{B/R}}$	Prob of suppressive interaction between mature APCs and primed T_{reg} cells	$[p_{\mathrm{R/B}}]^t$	Assumption: transpose of analogous reaction in the reverse direction
$p_{\mathrm{TC/R}}$	Prob of suppressive interaction between primed T cells and primed T_{reg} cells	0.5	Assume same as $p_{\mathrm{PK/x}}$ and $p_{\mathrm{A/\alpha}}$ in Table 5
c	Decay due to negative signaling	$1\ (\mathrm{k}/\mu\mathrm{L})^{-1}$	Estimation

5. Results

In this section we study the behavior of the model we developed in the previous sections. Our focus in on the role of regulatory cells in maintaining self-tolerance in the system. We start in Section 5.1 with examples of an over- under- and well-regulated immune response. In all cases, we consider two antigens simultaneously, one being foreign (or virus-infected) and the second being a self cell population. A well-regulated immune response can differentiate between the two target populations, eliminating the virus-infected population and preserving the normal self-population.

We then proceed in Section 5.2 to study the details of the dynamics of the various cell populations that are included in the model. Our main interest is in studying the different states of CD8+ T cells.

Finally, in Section 5.3 we apply LHS to analyze the dependence of the model's behavior on effector and regulatory reactivities. The analysis shows that the system can tolerate slightly self-reactive T cells that are monitored by regulatory T cells. More specifically, for a well-regulated immune response, we show that the effector reactivities should be only slightly higher than the regulatory reactivities.

We would like to comment on the calculation of the total number of activated T cells. We recall that the variables P_K, Q_K, and S_K denote the concentrations of primed, non-proliferating, and suppressed CD8+ cells, respectively. Also, there is no variable denoting the concentration of cells in the process of dividing. Rather, cells that commit to n rounds of division, exit the system at rate $p_{\mathrm{PK/x}}kx^L P_K^L$ and reenter the system $n\tau$ units of time later, (see (3.14) and (3.15)). Hence, the total concentration at time t of dividing cells is the integral of the rate they commit to division over the time interval $[t-n\tau, t]$. In particular, the concentrations of primed CD8+ cells in the process of dividing are

$$D_K = \int_{t-n_{PK}\tau}^{t} p_{\mathrm{PK/x}} f(y(u)) k g_+(u) P_K(u)\,\mathrm{d}u,$$

and the total concentrations of differentiated (i.e. non-naïve) CD8+ cells are

$$\text{Total CD8+ conc.} = P_K + Q_K + S_K + D_K.$$

Similarly, the concentrations of primed CD4+ cells in the process of dividing are

$$D_H = \int_{t-n_{PH}\tau}^{t} k\mathscr{J}(p_{\mathrm{TC/B}} f(y(u)), A_{\mathrm{Mij}}(u)) P_H(u)\,\mathrm{d}u,$$

and the total concentrations of differentiated (i.e. non-naïve) CD4+ cells are

$$\text{Total CD4+ conc.} = P_H + S_H + D_H.$$

All simulations in this section are done using the DDE solver 'dde23.m' from Matlab 7.0.

5.1. Over-regulated, under-regulated, and well-regulated immune responses

The model simulates the simultaneous immune response to two populations, which we label as the virus-infected and the normal self-populations. The virus-infected population, T_2^T, multiplies at a net growth rate of 0.2/day and starts in the tissue at a concentration of $1\,\mathrm{k}/\mu\mathrm{L}$ at time 0, (i.e. its initial history is $T_2^T(t)=0$ for $t<0$ and 1 for $t=0$. On the other hand, the normal self-population T_1^T has a net death rate of 0.01/day and is supplied into the tissue at a constant rate of 0.1 $(\mathrm{k}/\mu\mathrm{L})$/day. Hence, in the absence of a self-reactive immune response, the self-population remains at an equilibrium of $10\,\mathrm{k}/\mu\mathrm{L}$, so we set the initial history to be $T_1^T(t)=10$ for $t\leqslant 0$.

The three scenarios during an immune response are that the immune system is over- under-or well-regulated. The foreign-reactive regulatory probabilities are the only parameters that change in the following three examples. An example of an over-regulated immune response is shown in Fig. 9. The parameter values used for this example are listed in Appendix D. In the over-regulated immune response, the regulatory cells multiply and overpower the effector response, rendering the system tolerant to both virus-infected and normal self-populations. Thus, the self-population is spared as desired, but the virus-infected population never gets eliminated and relapses. Here, we set the self-reactive-regulatory probability to 0.3 and the foreign-reactive regulatory probability to 0.4. In Fig. 9, the CD4+ concentrations are very low because regulatory cells almost completely suppress CD4+ proliferation. As a result, the CD8+ T cells do not receive adequate positive signals to effectively proliferate.

An example of an under-regulated immune response is shown in Fig. 10. In the under-regulated immune response, we keep the self-reactive regulatory probability at 0.3 and set the foreign-reactive regulatory probability to 0. In this setup, regulatory cells do not multiply sufficiently to suppress the foreign-reactive CD4+ T cells. The foreign-reactive CD4+ T cells proliferate excessively which, in turn, stimulates self-reactive CD8+ T cells to proliferate due to the large increase in positive signaling. As a result, self-reactive effector cells end up destroying 70% of the self-population before the immune response subsides.

Since the normal concentration of T cells in the lymph node is about $500\,\mathrm{k}/\mu\mathrm{L}$, (see the estimates in Section 4.1), a CD8+ peak of $3000\,\mathrm{k}/\mu\mathrm{L}$ may be unrealistically high. At this point, other limiting factors such as limited resources other than IL-2 or space constraints will probably stop the T cells from proliferating.

Note that in Fig. 10 the self-reactive CD4+ concentration in the lymph node is very low. The autoimmune response results from the excess positive signal supplied by foreign-reactive CD4+ cells, rather than from self-reactive CD4+ cells. The overwhelming, foreign-reactive response elicits the bystander autoimmune response, implying that

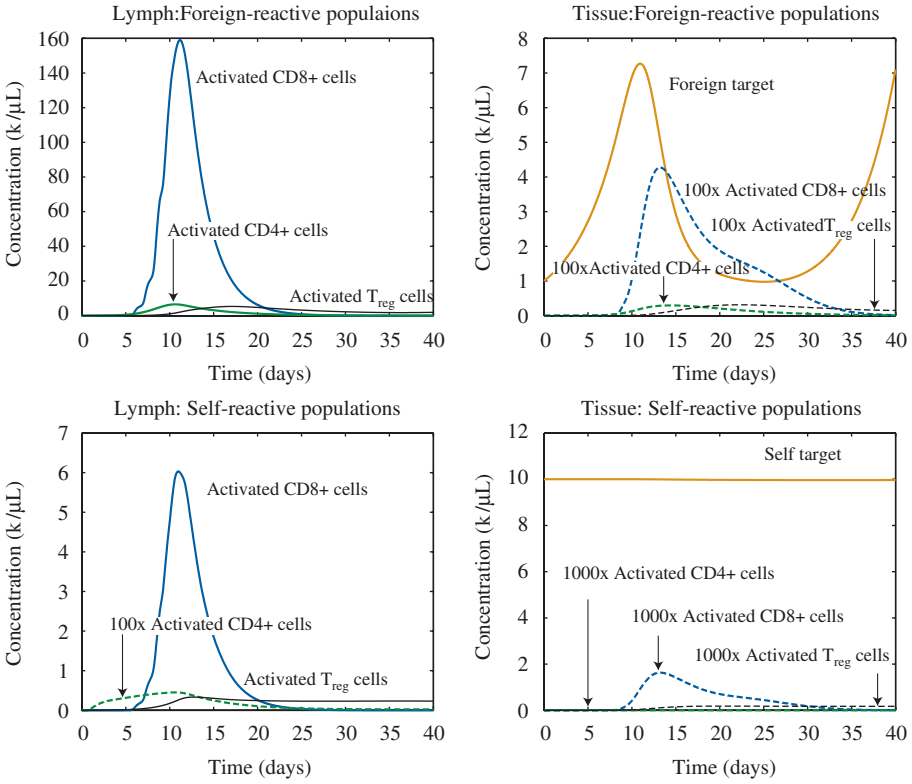

Fig. 9. Over-regulated immune response. The first and second columns show concentrations of cells or molecules in the lymph node and tissue, respectively. The top and bottom rows show concentrations of foreign and self-reactive populations, respectively. The concentrations of non-specific cells and molecules such as positive and negative signals are duplicated in both rows. In reality all populations are simultaneously present in the two-compartment system, but the plots are easier to view if separated into subsets.

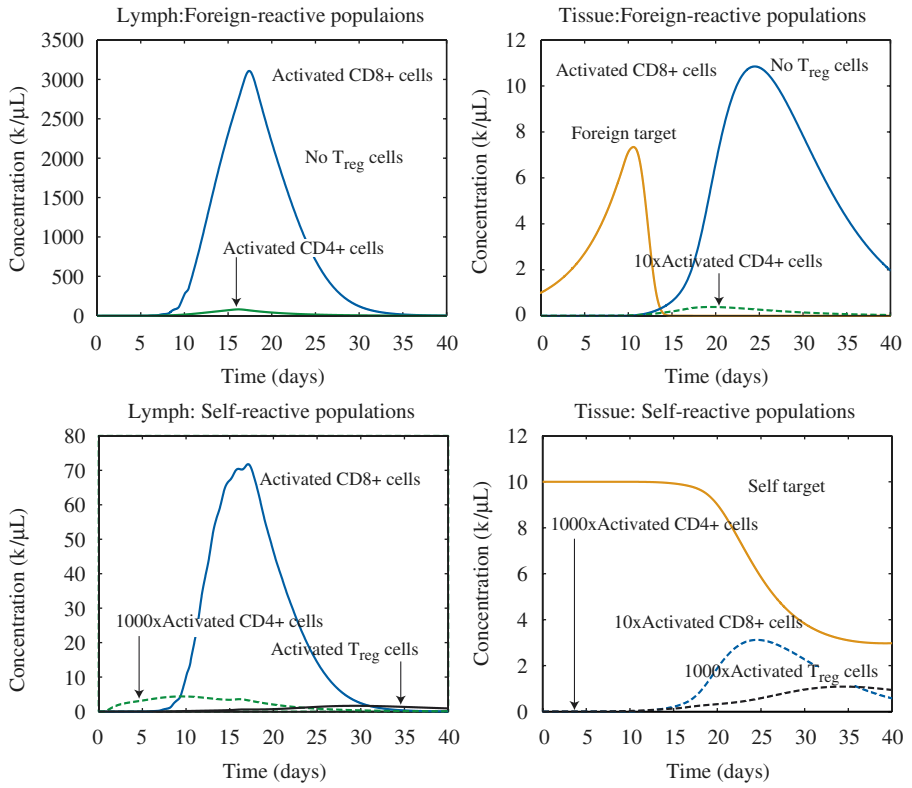

Fig. 10. Under-regulated immune response.

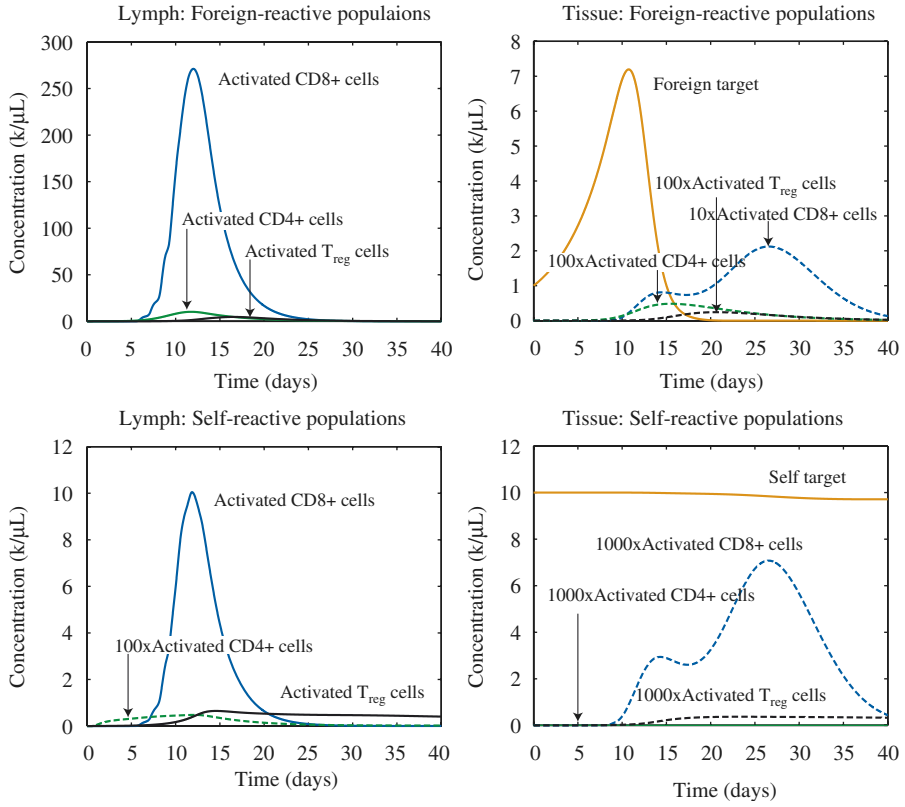

Fig. 11. Well-regulated immune response.

the regulation of the primary response is just as important as the regulation of bystander responses.

An example of a well-regulated immune response is shown in Fig. 11. Here, we keep the self-reactive regulatory probability at 0.3 and set the foreign-reactive regulatory probability to 0.3. The well-regulated response attains a balance between effector and regulatory cells that allows CD8+ T cells to destroy the virus-infected population, while sparing the self-population from excessive collateral damage.

It is not clear whether highly foreign-reactive regulatory cells exist, although such effects may come about via cross-reactivities of regulatory cells with foreign antigens. Hence, we show three analogous examples for which there are no foreign-reactive regulatory cells. Figs. 12 and 13 show examples of over-regulation and well-regulation, and Fig. 10 is already an example of underregulation without foreign-reactive regulatory cells.

The examples in Figs. 11 and 13 show that regulation is possible with or without foreign-reactive regulatory T cells. As expected, foreign-reactive regulatory cells are not required for regulation. However, their presence may modulate the dynamics of the immune response.

5.2. Detailed dynamics

It is instructive to view the dynamics of the various cell populations in detail. Figs. 14 and 15 show the details of the well-regulated immune response corresponding to Fig. 13.

Fig. 14 focuses on the CD8+ cells and on the positive and negative signals in the lymph node. The global dynamics are shown on the left-hand side. The right-hand side figures zoom on the region of low concentrations and initial times. Note that the population of primed CD8+ cells oscillates. This represents frequent transitions between the primed and dividing states, which occur in the presence of abundant positive signal. Although the primed population fluctuates, the population of total activated CD8+ cells rises and falls steadily. In a similar fashion, the positive signal level oscillates due to the fluctuation of primed CD8+ cells that consume the signal. We stress that the dynamics of the total number of CD8+ cells is very stable.

Fig. 15 shows the CD8+ populations in the tissue. In the lymph node most of the activated CD8+ cells are suppressed at a given time, whereas in the tissue most CD8+ cells are functioning as cytotoxic effectors. This phenomenon occurs because most of the regulatory cells remain in the lymph node, where they were originally activated.

Furthermore, suppressed CD8+ cells do not consume positive signal and initiate division, so they are more likely to flow out of the lymph node. In our model, we implicitly assume that cells in the process of dividing, stop interacting with the system during division. This assumption is

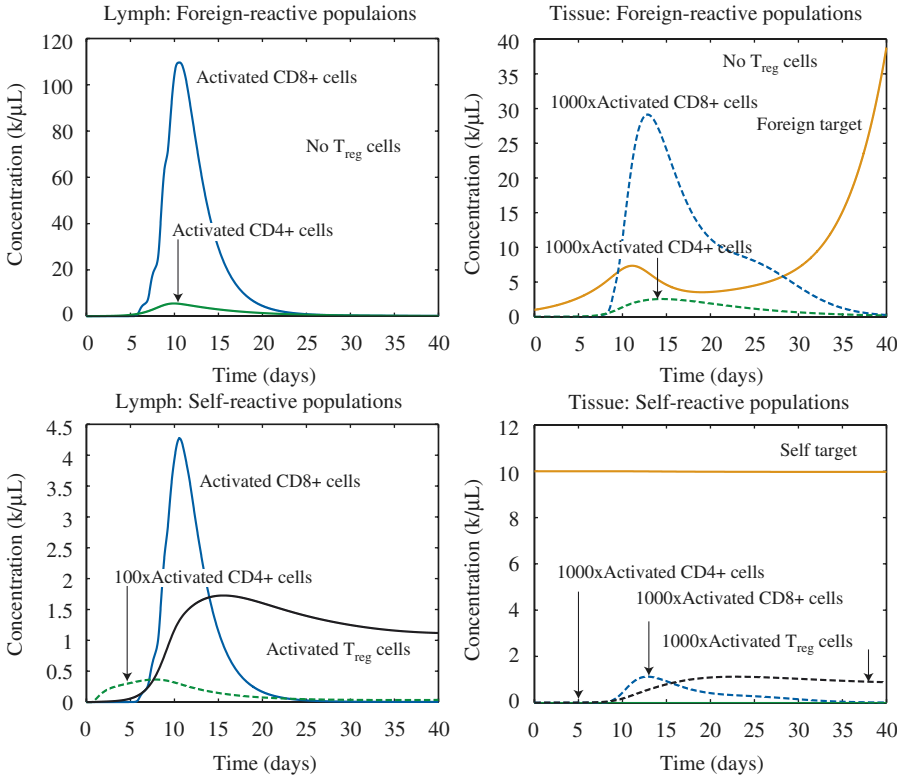

Fig. 12. Over-regulated immune response without foreign-reactive regulatory cells. Self-reactive regulatory probability is 0.8.

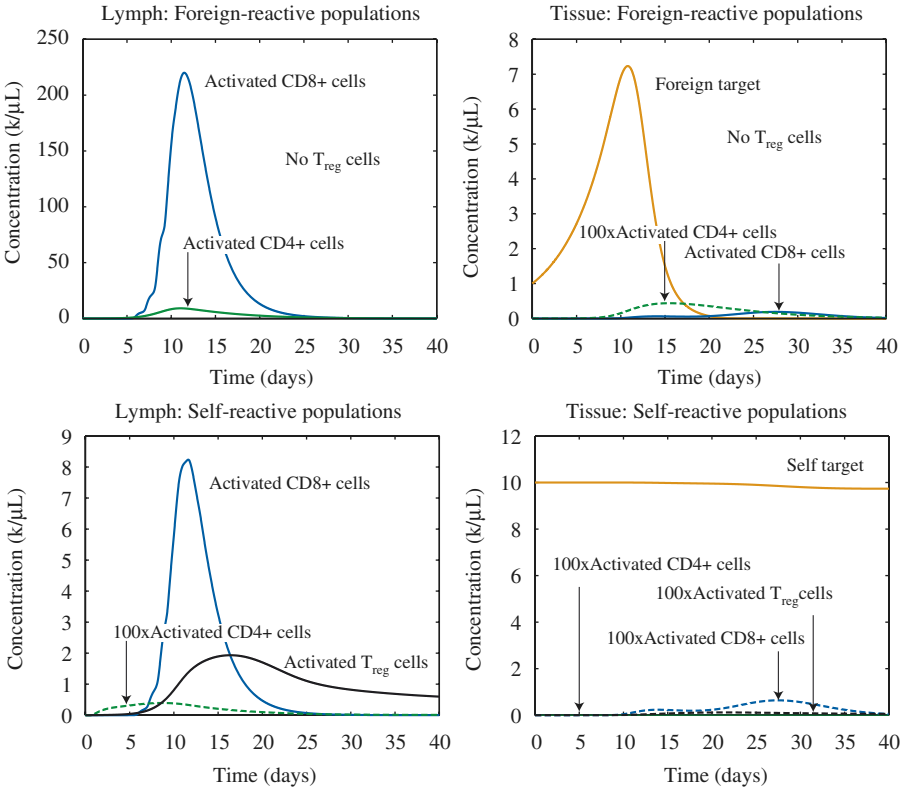

Fig. 13. Well-regulated immune response without foreign-reactive regulatory cells. Self-reactive regulatory probability is 0.6.

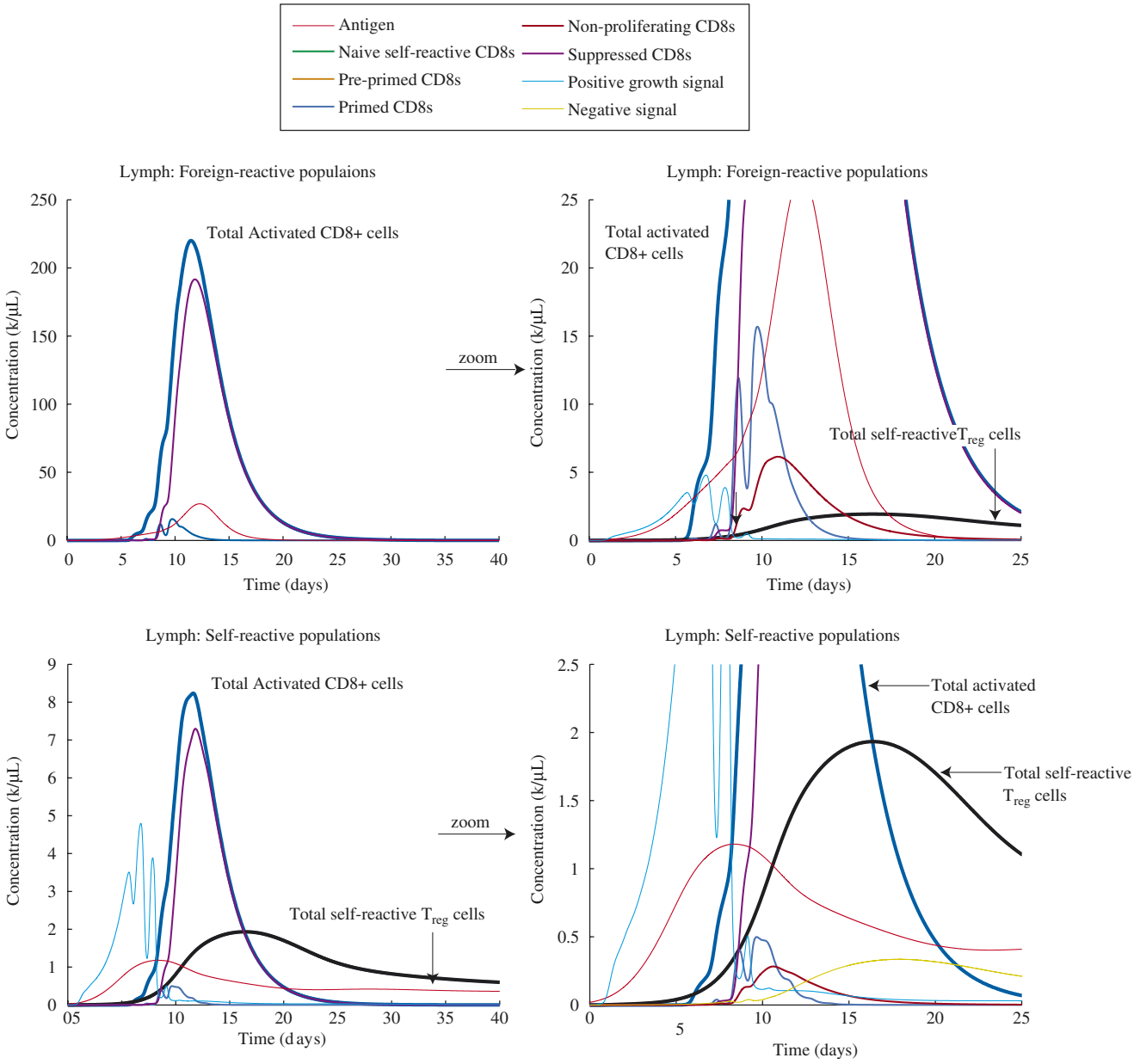

Fig. 14. CD8+ cells and signals in lymph.

included in the formulation of the equations for primed T cells in Section 3, since dividing cells exit the system at a certain rate $R(t)$ and reenter the system $n\tau$ time units later at rate $2^n R(t-n\tau)$. During division a cell also will not migrate from the lymph node to the tissue compartment or vice versa.

Thus, by halting division, regulatory cells induce a population contraction and emigration to the tissue. Indeed, Fig. 15 shows that CD8+ cells start actively migrating to the tissue at about day 20, while Fig. 14 shows that before day 20, CD8+ cells remain sequestered in the lymph node for a phase of rapid proliferation. In addition, the timing of the regulatory cells peak falls between the CD8+ peaks in the lymph node and tissue.

This two-phase cycle of lymphocyte sequestration and proliferation followed by contraction and emigration is shown in Figs. 9–13. These results suggest that over-regulation may occur from a premature transition to the contraction and emigration phase, while underregulation results from a belated transition. Proper regulation occurs when the CD8+ cells have sufficient time to expand in the lymph node before emigrating to the tissue.

Fig. 15 also shows the dynamics of the two target populations and antigens in the tissue. Notice that the self antigen concentration rises slightly as the self-population dips. This is because dying cells release antigen, which may stimulate an increased anti-target response in a positive feedback loop. In this case, however, regulation prevents

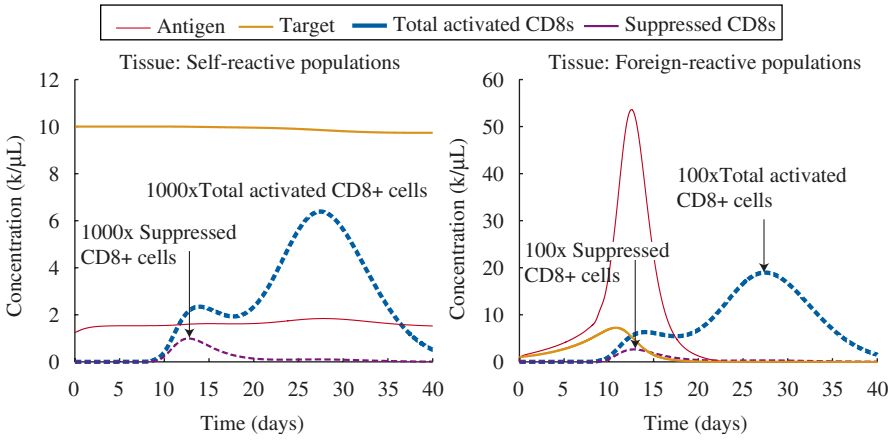

Fig. 15. Target, antigen, and CD8 cells in tissue.

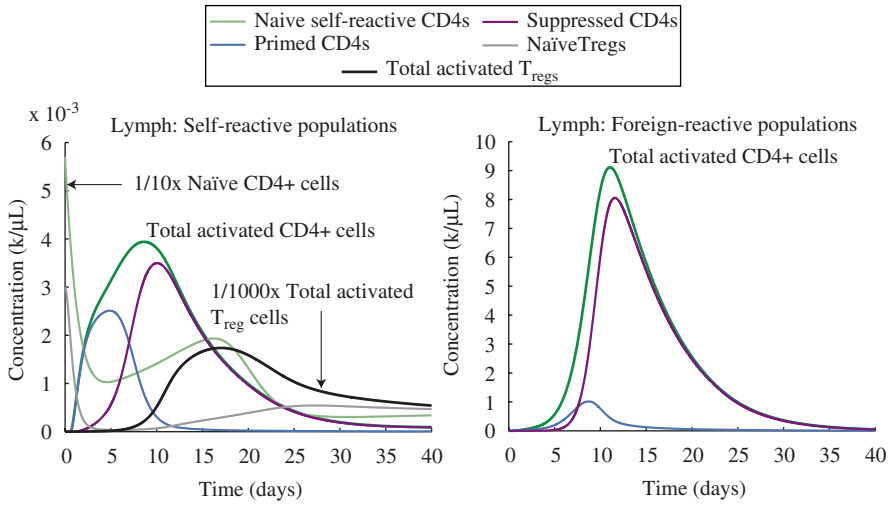

Fig. 16. CD4+ cells in lymph.

the self-population from being destroyed. On the other hand, the concentration of foreign antigen rises and falls rapidly, magnifying the fluctuation of the virus-infected cell concentration. The slight increase of foreign antigen due to elimination of virus-infected population is not as noticeable as in the case of self antigen.

Fig. 16 shows the fluctuations of CD4+ cells in the lymph node. The dynamics of the CD4+ populations follow the same pattern as the CD8+ populations, although their peaks are roughly 25 times lower in the case of foreign-reactive CD4+ cells or 2×10^3 times lower in the case of self-reactive CD4+ cells. Thus, self-reactive CD8+ cells are able to proliferate despite there being hardly any primed self-reactive CD4+ cells. This is a consequence of the fact that self-reactive CD8+ cells can proliferate from the positive signal supplied by foreign-reactive CD4+ cells, whereas self-reactive CD4+ cells are directly suppressed by immune regulation.

Fig. 17 shows the concentrations of APCs in the lymph node. In the presence of high inflammation, the APCs collect circulating antigens and transition from immature to mature states. As expected, foreign-antigen-bearing APCs remain in the lymph node as long as virus-infected population is present in the tissue.

5.3. Balancing the effector and regulatory reactivities

To study the effects of various parameters on the model behavior, we use LHS (McKay et al., 1979). This method involves numerically solving the system of equations multiple times with randomly sampled parameter values. The samples are chosen such that each parameter is well distributed over its range of possible values.

In the present study, we vary four groups of parameters:

1. Anti-self effector reactivity:
   - Upper-left elements of the 2×2 matrices: $p_{TC/B}$, $p_{A/PH}$, $p_{PK/T}$.
2. Anti-foreign effector reactivity:
   - Lower-right elements of the 2×2 matrices: $p_{TC/B}$, $p_{A/PH}$, $p_{PK/T}$.
3. Anti-self T_{reg} reactivity:
   - Upper-left elements of the 2×2 matrices: $p_{R/B}$, $p_{B/R}$.

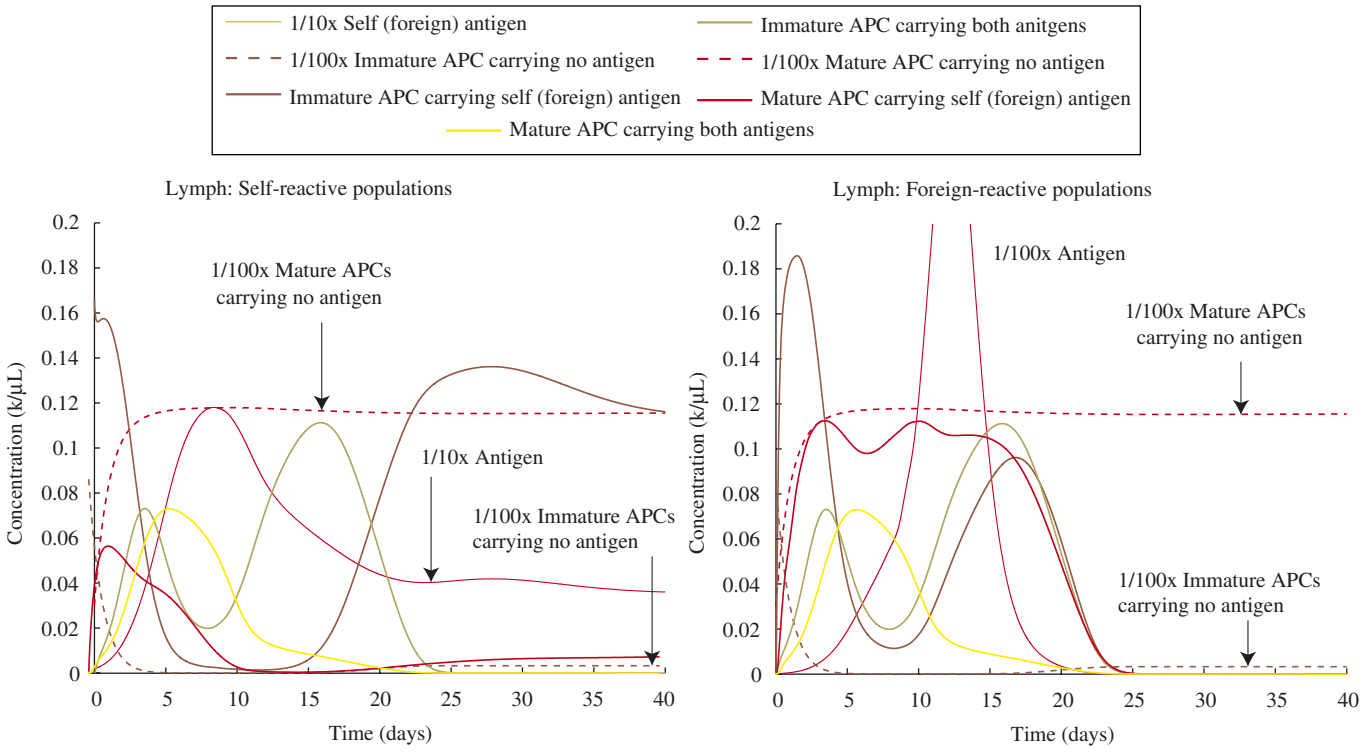

Fig. 17. APCs in lymph.

4. Anti-foreign T_{reg} reactivity:
   - Lower-right elements of the 2×2 matrices: $p_{R/B}$, $p_{B/R}$.

We vary the four parameter groups according to a uniform probability distribution in the interval $[0, 1]$.

Since the four groups are varied randomly, it is possible that either the CD8+ cell count or the foreign population will blow up. We therefore use the following stopping criterion for the simulations:

- A primed CD8+ populations in the lymph node, $P^L_{K,1}$ or $P^L_{K,2}$, exceeds $1000\,\mathrm{k/\mu L}$.
- A target population in the tissue, T^T_1 or T^T_2, exceeds $100\,\mathrm{k/\mu L}$.

The simulations are evaluated over a time interval of up to 40 days.

For each set of parameters, we associate the results of the corresponding simulation with one of the following categories:

1. *Over-regulated*: The primed T cell populations never exceed $1000\,\mathrm{k/\mu L}$, self-population is tolerated, and foreign population survives (i.e., $T^T_2(40) \geqslant 4\times 10^{-5}\,\mathrm{k/\mu L}$).
2. *Under-regulated*: One of the primed T cell populations reach $1000\,\mathrm{k/\mu L}$, or the self-population is not tolerated (i.e., $\min_{t\in[0,40]} T^T_1 \leqslant 0.5$).
3. *Well-regulated*: The primed T cell populations never exceed $1000\,\mathrm{k/\mu L}$, the self-population is tolerated, and the foreign population is eliminated (i.e., $T^T_2(40) < 4\times 10^{-5}\,\mathrm{k/\mu L}$).

We now establish criteria for whether a population is considered tolerated or eliminated.

We consider the self-population tolerated by an immune response, if its minimum concentration in the tissue is above 50% of its equilibrium value, (i.e., $\min_{t\in[0,40]} T^T_1 > 0.5$). Recall that in Section 4.1, we calculated the volume of the hypothetical lymph node to be $4.2\,\mu\mathrm{L}$. Also, recall from Table 2 that we estimate the volume ratio between the tissue and lymph node to be 6. Hence, we estimate a tissue volume of $25.2\,\mu\mathrm{L}$, and so the concentration of one cell in the tissue is $(1/25.2)\,\mathrm{cells/\mu L} = 4 \times 10^5\,\mathrm{k/\mu L}$. Thus, our criterion for foreign cell elimination is that its end time population is less than $4 \times 10^{-5}\,\mathrm{k/\mu L}$, (i.e., $T^T_2(40) < 4 \times 10^{-5}\,\mathrm{k/\mu L}$). Note that if a run is stopped prematurely due to the stop criterion, we consider the system over-regulated if the foreign population has exceeded $100\,\mathrm{k/\mu L}$, and we consider the system under-regulated if either of the primed CD8+ populations has exceeded $1000\,\mathrm{k/\mu L}$.

The results for 100 LHS simulations are shown in Fig. 18. As the plots show, only two of the sampled parameter combinations yield well-regulated responses. Hence, with the current parameter distribution, the cytotoxic immune response tends to be too strong,

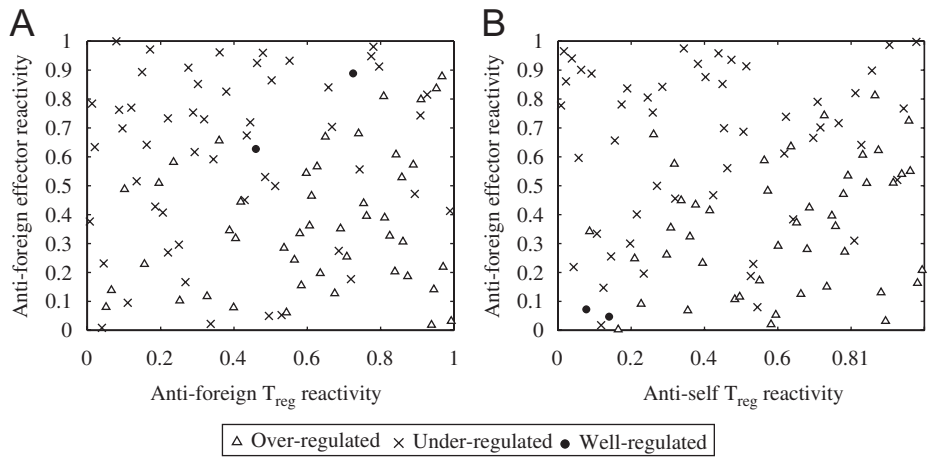

Fig. 18. Outcomes of 100 LHS simulations. The four parameter groups, self and foreign-reactive effector reactivities and self and foreign-reactive T_{reg} reactivities vary uniformly between 0 and 1.

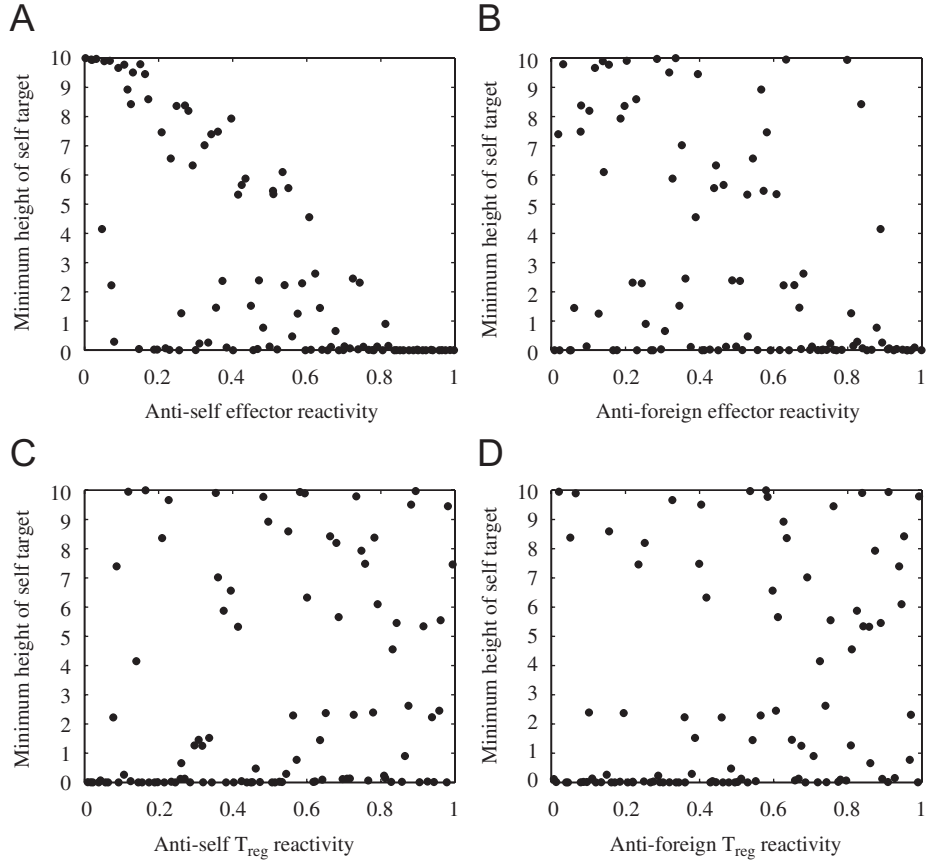

Fig. 19. Minimum heights of self concentration versus the four parameter groups.

resulting in excessive collateral damage while eliminating foreign population.

To determine which parameter group has the greatest effect on the level of collateral damage we plot the minimum height of the self concentration against the four parameter groups in Fig. 19. From the four plots, it appears that the anti-self effector reactivity has the greatest influence on the collateral damage to the self-population. In fact, a steep increase in the level of collateral damage begins as the anti-self effector reactivity passes beyond ~0.1. Hence, it appears that a well-regulated immune system cannot depend on peripheral T cell regulation alone, but must ensure that potentially self-reactive T cells are not too self-reactive, as expected. Thus, in accordance

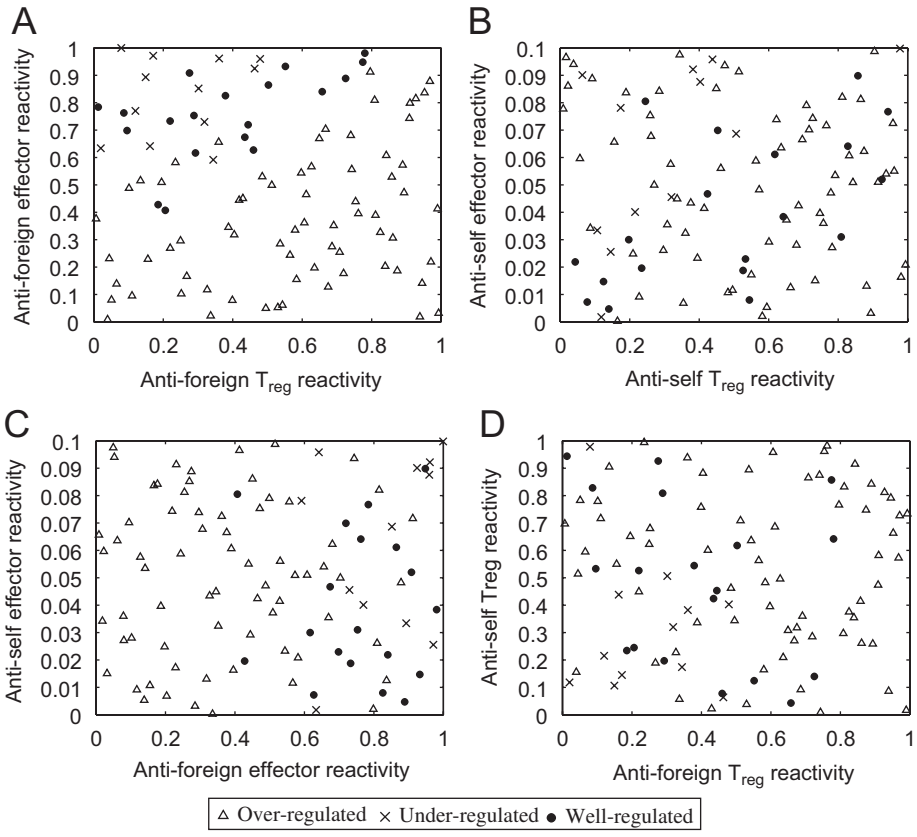

Fig. 20. Outcomes of 100 LHS simulations. The three parameter groups, foreign-reactive effector reactivity and self and foreign-reactive T_{reg} reactivities vary uniformly between 0 and 1. The self-reactive effector reactivity varies uniformly between 0 and 0.1.

with accepted biological hypotheses, the model implies that a stable system requires a method of central tolerance, like the one mediated by the thymus, in conjunction with peripheral tolerance, mediated by regulatory T cells.

To generate a more useful data sample, we perform another LHS simulation where we limit the range of self-reactive effector probabilities to a uniform distribution between 0 and 0.1. The results for 100 runs is shown in Fig. 20. In this case 19 of 100 simulations are well-regulated. Fig. 20A, shows the most clear clustering of well-regulated responses. We interpret this observation as suggesting that the two most important parameters for obtaining a well-regulated response are the anti-foreign effector and regulatory reactivities. Furthermore, notice that even the over- and under-regulated clusters in this image are fairly separated, with the well-regulated region lying between them. This implies that there is an optimal balance between effector and regulatory reactivities.

The other three plots, Fig. 20B–D, do not possess such clear regions of well-regulated simulations, but Fig. 20B and C show that lower self-reactive effector probabilities are beneficial. Fig. 20D shows that higher self and foreign-reactive regulatory probabilities are both important for a well-regulated system, but foreign-reactive regulatory cells are slightly more important.

We also consider the case where regulatory cells are exclusively self-reactive and see that such a system has a similar behavior to the system shown in Fig. 20. For these LHS simulations, we used the same parameters as in Fig. 20 but set the values of the foreign-reactive regulatory probabilities to 0. The results for 100 runs are shown in Fig. 21. Here, 17 of 100 simulations are well-regulated. The separations between the regions of over-, under-, and well-regulated immune responses in Fig. 21A is quite clear. Similarly to the previous simulation, this study also implies that the two most important parameters are the anti-foreign effector and anti-self regulatory reactivities.

5.4. Contact-dependent and cytokine-related regulatory mechanisms

Regulatory cell functions can be grouped into two categories: cytokine-related and contact-dependent behavior. Although we leave a thorough sensitivity analysis for a future work, in this paper, we examine the relative contributions of these two regulatory mechanisms.

To test the significance of the consumption of positive signal and the secretion of negative signal by T_{reg} cells (regulatory mechanisms 4 and 5 in Fig. 8), we take the parameters from the well-regulated system in Fig. 13 and set the consumption rate of positive signal and the secretion rate of negative signal by T_{reg}s to 0. See Fig. 22.

Note that there is hardly any difference in the impact on the normal self population. To be precise, the self

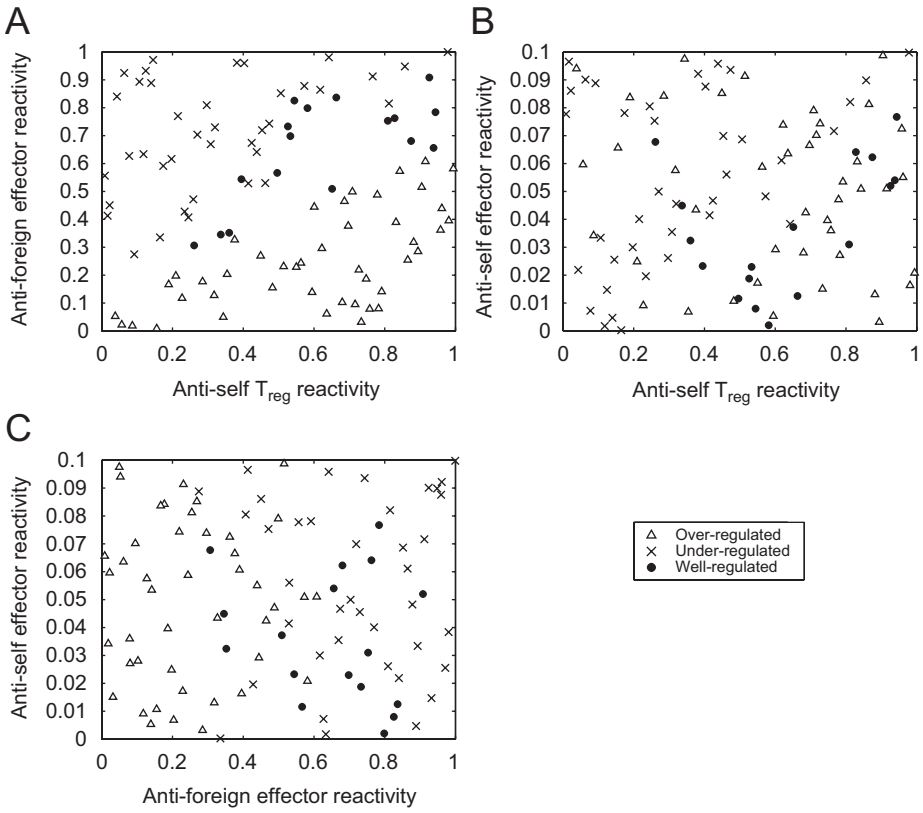

Fig. 21. Outcomes of 100 LHS simulations. The foreign-reactive effector reactivity and self T_{reg} reactivity vary uniformly between 0 and 1. The self-reactive effector reactivity varies uniformly between 0 and 0.1. The foreign-reactive T_{reg} reactivity is 0.

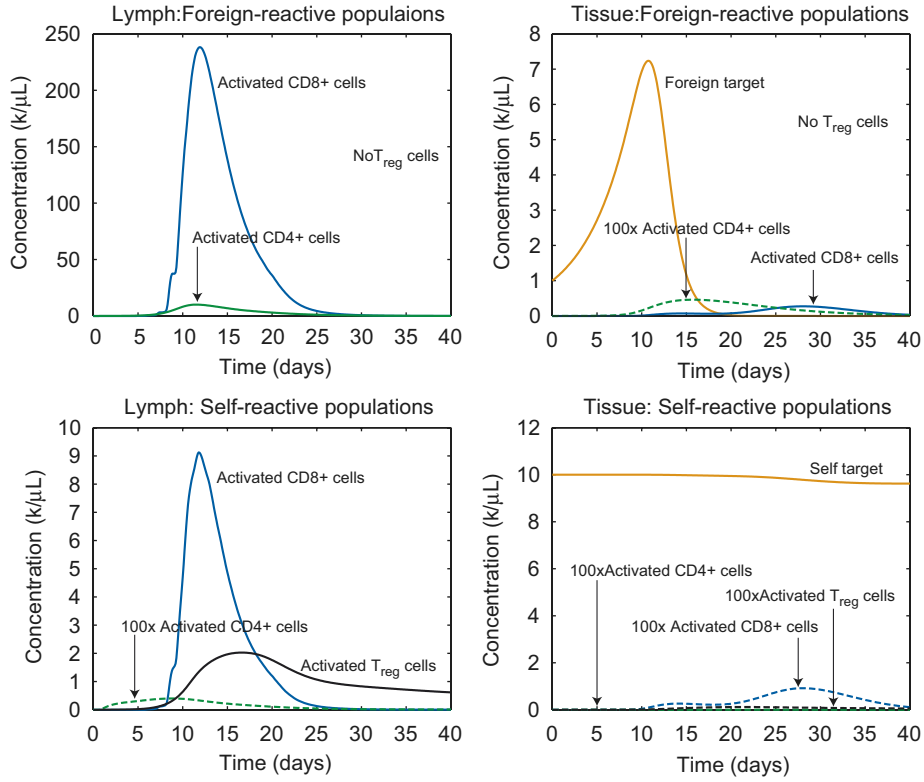

Fig. 22. Time evolution of cell populations with same parameters as in Fig. 13, except that the secretion rate of negative signal by T_{reg} cells is set to 0.

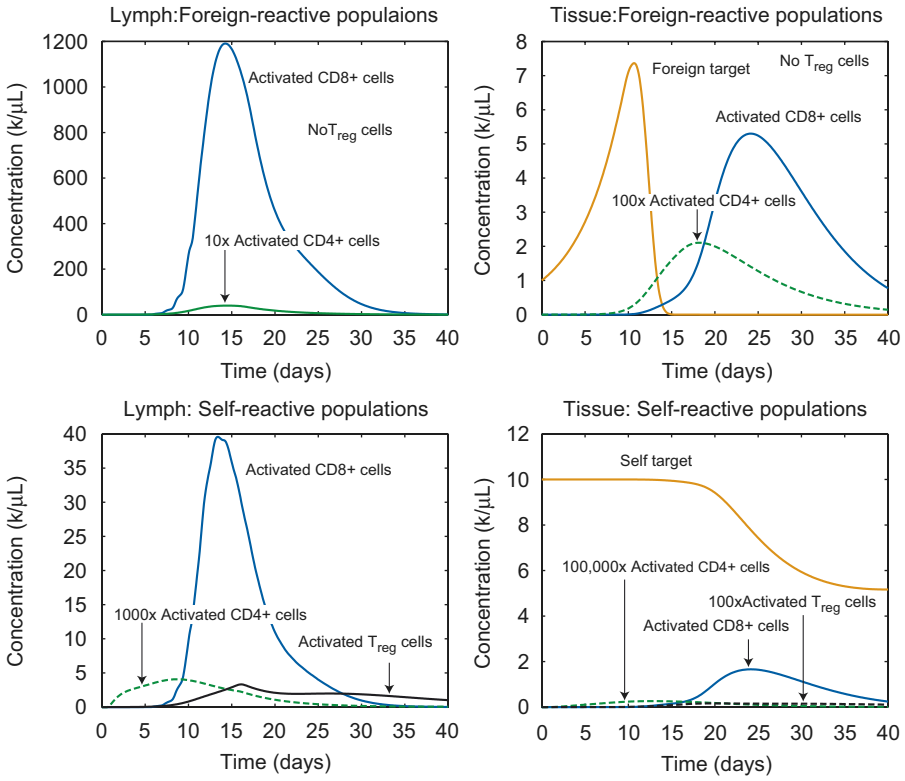

Fig. 23. Time evolution of cell populations with same parameters as in Fig. 13, except that the probability of interaction between T_{reg} cells and immune cells (anti-self regulatory reactivities in Table D4) is set to $40\% \times 0.6 = 0.24$.

population in Fig. 13 drops to 9.73 k/μL, while the normal self population in Fig. 22 drops to 9.62 k/μL. Hence, the difference is about 1%.

To test the significance of the contact-dependent T cell suppression (regulatory mechanisms 1 and 2 in Fig. 8), we take the parameters from the well-regulated system in Fig. 13 and set the probability of T cell suppression by regulatory cells (parameter $p_{TC/R}$ in Table D3) to 30% of the value in Fig. 13. See Fig. 23. We stop at 30% rather than going on to a total knockout, because below 30%, the CD8+ T cells in the lymph node proliferate too quickly, making an accurate numerical simulation impractical.

In Fig. 23, about 50% of the normal self population is destroyed during the course of the immune response. Note that the anti-foreign CD8+ T cell concentrations of 1.2 million cells per μL in the lymph node in Fig. 23 is higher than usual. At this point, other restrictions, such as limited resources, would hinder the excessive expansion of T cells; however, this simulation demonstrates that the contact-dependent regulatory mechanisms of regulatory cells greatly influence T cell proliferation.

6. Discussion

The dichotomy between self and non-self antigen has been evolving over the past several years. Whereas the classical paradigm asserts a strong schism between purely self and purely non-self antigens, more recent studies favor the viewpoint that many T cells are slightly cross-reactive to an array of different antigens, including common self antigens (Sakaguchi et al., 1995). This implies that although TCR are highly specific, their specificity is not black and white. Consequently, the risk of autoimmune disease is always present and may arise from inadvertently stimulating and expanding slightly self-reactive effector T cells.

Autoimmunity may occur from bystander stimulation where a robust immune response against foreign antigen leads to a flood of mature APCs and positive growth signal into a lymph node, causing dormant effector T cells to start responding against self antigen. In León et al. (2004), the authors have used their results from León et al. (2000, 2001) to study how infections may cause an immune system to become autoimmune via bystander activation. Alternatively, the T cells that are part of the initially anti-foreign immune response may also be slightly self-reactive, leading to a lingering autoimmune response. In this paper, we focus on the first scenario of a bystander stimulation. To study the second scenario, we need to consider one population of T cells that is strongly reactive to the foreign population and slightly cross-reactive to the self. We save this study for a future work.

In the results shown in Section 5, the presence of a foreign antigen sets off a vigorous immune response. The naturally-occurring regulatory response is initiated at about the same time as mature APCs flood the lymph

node. In this model, the regulatory response is almost always slower because they begin at a lower precursor frequency and are less reactive than the anti-foreign effectors. If some regulatory cells are reactive to the foreign antigen, this regulation ramps up on the same stimulus as the effector response.

On the other hand, if there are only self-reactive regulatory cells, self antigen needs to be available at sufficient levels on mature APCs to activate self-reactive regulatory cells. Since most self antigens are mostly ubiquitous, they will likely be present in large numbers. However, this might not occur if there is a small population of normal self cells, presenting a rare self antigen. In this case, the self-reactive regulatory response may initiate too slowly to prevent major collateral damage to the self population. In particular in this model, if we take the same parameters used in Fig. 13 and change the normal self target supply rate to $0.01\,\mathrm{k}/\mu\mathrm{L}$ per day and its initial concentration to the equilibrium value of $1\,\mathrm{k}/\mu\mathrm{L}$, there is almost no regulatory response to prevent a rapid bystander expansion of anti-self effector cells. On the other hand, if we take the parameters used in Fig. 11 and change the self target parameters as above, the foreign-reactive regulatory cells keep the immune response under control and keep the collateral damage to less than 10%.

Paradoxically, in the case where there are no foreign-reactive regulatory cells, it seems that a more intense infection that affects more or larger self populations will be better regulated. This possible inverse correlation between infections and autoimmunity was also discussed in the model of León et al. (2004).

For regulation to be effective, regulatory cells must be sufficiently activated in tandem with a strong effector response. If the regulatory cells respond to a different antigen (e.g. self-antigen) than the main effector response, the system must ensure that there is enough alternative antigen to simultaneously stimulate the regulatory cells. Since the dynamics of the model depends on whether the regulatory cell repertoire greatly overlaps, perhaps even contains, the effector repertoire, a useful study would be to see whether populations of naturally-occurring regulatory cells and effector cells can be expanded from a single blood sample with the same stimulating antigen.

The model incorporates many combinations of interactions between populations and requires the estimation of numerous parameters. A formal sensitivity analysis is left for a follow up paper, but we state several conclusions from our observations.

First, the primary regulatory mechanism is contact-dependent suppression. Negative signaling modulates the level of regulation and can enhance the ability of regulatory cells to avert an autoimmune response, but they are far from being sufficient on their own. The negative signaling parameter, c, was estimated without much information, but it would have to be very high (or negative signals would have to be secreted very fast by regulatory cells) for negative signaling to be a primary method of regulation.

Burroughs et al. (2006) analyze a model that looks more specifically at the effect of the inhibition of IL-2 secretion by regulatory cells. They conclude that such cytokine-dependent regulation adjusts the quorum threshold, i.e., the number of effector T cells that need to be activated to induce an immune response. However, they also observe that this method of regulation is prone to fail if previously dormant, autoimmune T cells are driven to expansion via cross-reactivity or bystander stimulation. The latter observation coincides with our conclusion that cytokine-dependent regulation alone is insufficient.

The suppression of CD4+ cells by regulatory cells is the main contact-dependent mechanism. CD4+ suppression controls the main positive feedback loop in which primed CD4+ cells stimulate immature APCs and mature APCs simulate CD4+ cells. This feedback loop drives the immune response by maintaining high levels of antigen presentation to other T cells, and unless it is halted, the immune response continues.

A primary mode of antigen presentation is APCs that collect antigen in the periphery and migrate to the lymph node. Antigen also drains into the lymph node, where it may be collected by immature APCs, which are then stimulated by primed CD4+ cells. However, this latter mechanism for antigen presentation is less effective and only picks up after the antigen-bearing APCs have immigrated from the periphery and already stimulated large numbers of CD4+ cells.

In our model, we assumed that effector cells become tolerogenic after interacting with immature APCs, while regulatory cells do not. We have no experimental basis for this asymmetry, and it is possible that immature APCs may also render regulatory cells ineffective. A study on the interaction between naturally-occurring regulatory cells and immature APCs would be useful, and may change the dynamics of the model.

The phenomenon that primed T cells remain sequestered in the lymph node during the initial phase of proliferation, depends on the assumption that dividing T cells do not migrate, or at least are less likely to migrate. This assumption is similar to saying that dividing T cells prefer to remain where there is more IL-2 or other positive signal. Another important assumption is that the suppressed state of T cells is not permanent, but reverses in the absence of regulatory cells. If suppression were permanent or long-lived, the T cells that emigrate from the lymph node would be useless against target cells.

Based on these assumptions, regulatory cells increase the efficiency of strong immune responses by halting the cycle of T cell division and causing T cells to migrate to the periphery. This implies that a regulated immune response can be about as effective against foreign targets as an unregulated response, but with much less proliferation of effector T cells. Preventing the excessive expansion of effector T cells is more efficient and also safer. Hence, regulation may be beneficial for strong cytotoxic T cell responses. In addition, a small population of

naturally-occurring foreign-reactive regulatory cells may be feasible and even advantageous.

If the two-phase cycle of lymphocyte sequestration and emigration exists, the advantage of temporarily confining primed T cells to the lymph node is that the primed CD4+ cells contribute to further stimulating immature APCs. Also, this confinement allows primed CD8+ cells to dedicate themselves to a period of rapid proliferation by remaining in the IL-2-rich environment created in the lymph node. This phenomenon would imply partly distinct recruitment and deployment phases whose transition is initiated by regulatory cells.

7. Conclusions

We have developed a mathematical model that simulates the dynamics of a T cell-mediated immune response and the dynamics of regulatory cells. The analysis shows that self-reactive T cells can exist and are controlled by regulatory cells. On the other hand, if regulation is too weak or absent, even the presence of weakly self-reactive T cells can result in autoimmune reactions. These secondary autoimmune responses may be initiated as bystander reactions to stronger primary immune responses against foreign cells.

By examining the population dynamics of immune cells closely, we observe that the CD8+ T cell response has two phases. During the first phase, CD8+ T cells are sequestered in the lymph node where they proliferate rapidly. In the following phase, regulatory T cells multiply and suppress T cell activity, which causes effector CD8+ cells to stop dividing and emigrate to the tissue, where these effectors eliminate target cells, whether foreign or normal self cells. Furthermore, the regulatory cell concentration in the lymph node is much higher than in the periphery, resulting in stronger immune cell suppression in the lymph node than the periphery during the latter stages of the immune response. In addition to controlling autoimmune reactions, regulatory cells may aid in propelling a timely immune response against foreign cells by inducing an emigration of CD8+ effectors from the lymph node.

Using LHS methods, we analyzed the dependence of the model's behavior on four parameters at once. We first concluded that peripheral tolerance mediated by regulatory cells is not sufficient to prevent autoimmune disease, but there must be a method of ensuring that self-reactive T cells are not too self-reactive. While it may be optimal, or even necessary, to allow some self-reactive T cells to survive within the immune repertoire, it should not include highly self-reactive T cells. This observation is in concordance with the mechanism of central tolerance, which positively or negatively selects T cells based on TCR reactivities to self antigen.

A well-regulated immune response, in which foreign cells are eliminated while normal self cells are tolerated, results from a balance between effector and regulatory reactivities. The optimal combination for anti-virus responses occurs when effector cells are slightly more reactive than regulatory cells. This balance ensures that effector cells can proliferate earlier and faster than regulatory cells, but also that regulatory cells can proliferate quickly enough to suppress over-proliferation of immune cells or bystander autoimmune reactions.

Our current results do not decisively indicate whether it is advantageous to have some foreign-reactive regulatory cells or not. Furthermore, it is possible that the generation of adaptive regulatory cells from persistently stimulated effector cells (Walker et al., 2005) may yield similar benefits as having naturally-occurring foreign-reactive regulatory cells.

In this paper, we determined the necessary balance between effector and regulatory reactivities, but did not suggest a viable mechanism for attaining this equilibrium. Another possibility is that effector and regulatory cells may arise via the same generative processes and have the same TCRs, but enter the system in different proportions depending on positive and negative selection in the thymus. This hypothesis proposes that positive and negative selection are not absolute, but a probabilistic processes that allow mixed populations of effector and regulatory cells to enter the system for each TCR-specificity.

This proposition that positive and negative selection may be stochastic was also examined in a mathematical model incorporating thymic selection and T cell regulation (León et al., 2003). In this paper, León et al. conclude that repertoire selection may be stochastic, but a sufficient number of self-reactive regulatory cells must be positively selected, while negative selection must be strong enough to prevent the introduction of highly self-reactive effectors.

In our modeling and analysis, we focused on the role of regulatory cells during a primary immune response, but as a future work, we can study how an immune system acquires tolerance after chronic encounters with a particular antigen.

Acknowledgments

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Appendix A. Comprehensive diagrams

See Fig. A1 for a comprehensive diagram, Fig. A2 for a comprehensive diagram of APC interactions, and Fig. A3 for a comprehensive diagram of T cell interactions.

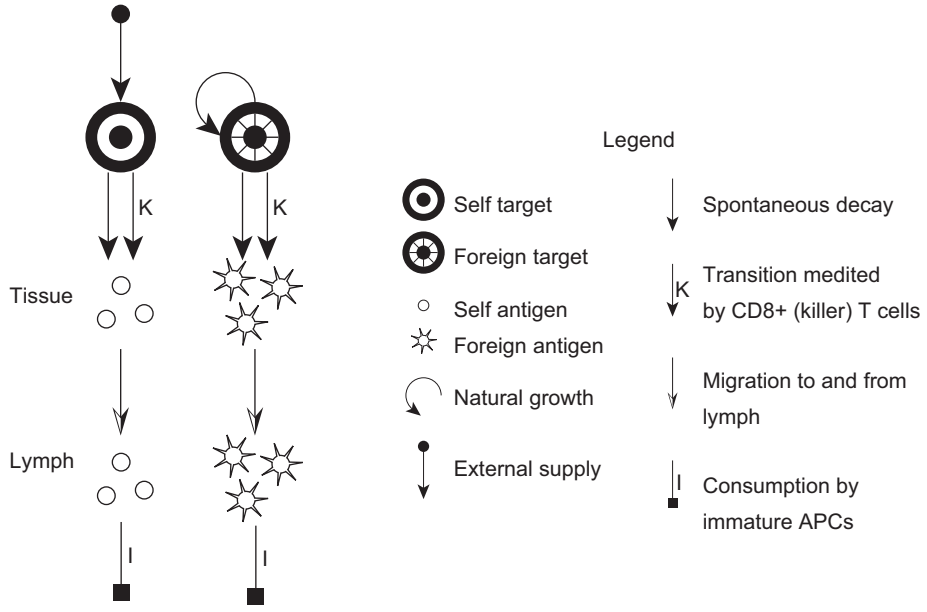

Fig. A1. Comprehensive target and antigen diagram. See Section 2.2 . (All antigen naturally decays at a constant rate, which is not shown. Target cells perish at a natural rate and shed antigen.)

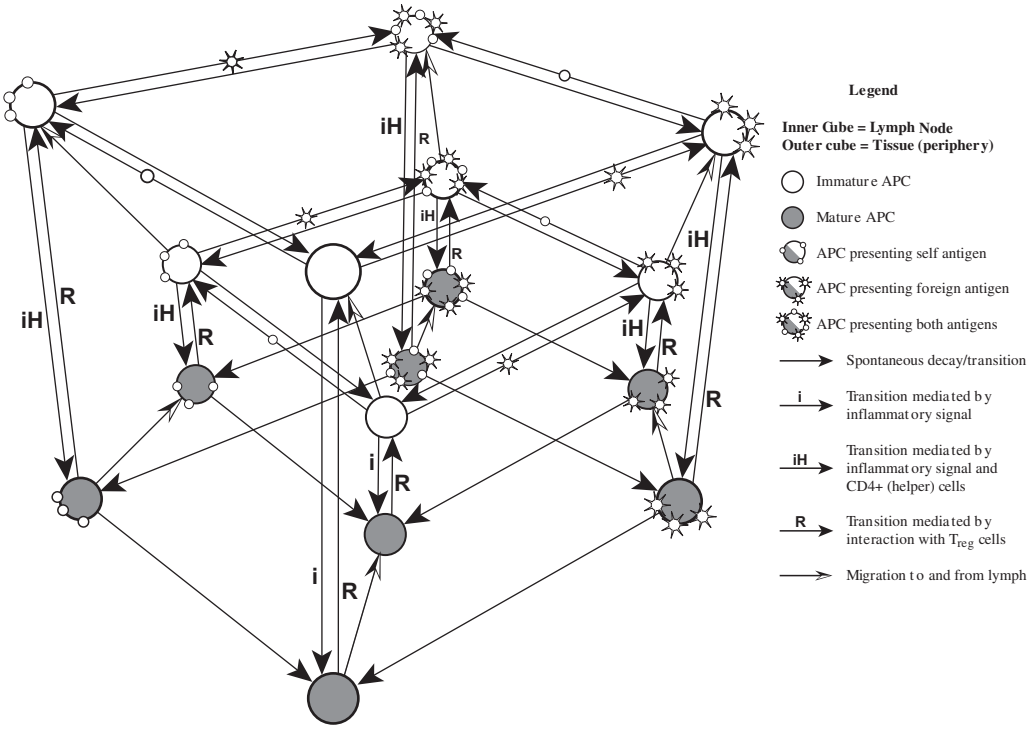

Fig. A2. Comprehensive APC diagram. The inner cube corresponds to the lymph node. The outer cube corresponds to the tissue. See Section 2.3. (Every cell perishes at a natural death rate, which is not shown.)

Appendix B. Derivation of the discounting factor

Suppose we have a one-variable system in which a substance flows in at rate $r(t)$ and flows out after τ units of time. Suppose we also have a continuous depletion (Fig. B1) rate of $d(t)X(t)$, where $X(t)$ represents the amount of substance in the system at time t.

Since there is depletion throughout the time period $t-\sigma$ to t, the outflow rate at time t should be less than $r(t-\sigma)$.

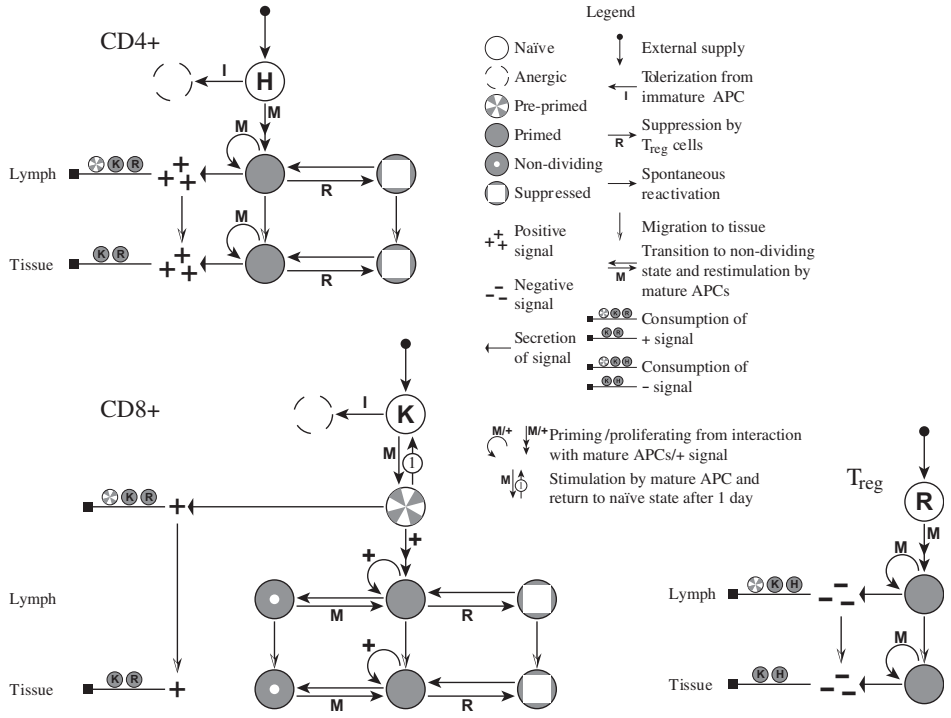

Fig. A3. Comprehensive T cell diagram. H, K, and R denote CD4+ (helper), CD8+ (killer), and regulatory T cells, respectively. See Sections 2.4–2.6. (Every cell perishes at a natural death rate, which is not shown. Furthermore, all active T cells take up negative signal, which decreases their reaction probabilities. See Section 3.10.)

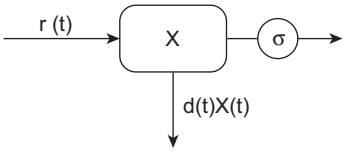

Fig. B1. Continuous depletion.

Let $F(s,t)$ represent the amount of substance remaining at time s that will exit at time t. Then, the substance entered at time $t-\sigma$, so $F(t-\sigma,t)=r(t-\sigma)$. If we assume that the depletion rate is uniform over the whole amount X, then

$$\frac{\partial F(s,t)}{\partial s}=-d(s)F(s,t).$$

Hence, by separation of variables,

$$F(s,t)=\mathrm{e}^{-\int_{t-\sigma}^{s}d(u)\,\mathrm{d}u}F(t-\sigma,t)=\mathrm{e}^{-\int_{t-\sigma}^{s}d(u)\,\mathrm{d}u}r(t-\sigma),$$

so

$$\begin{aligned}\frac{\mathrm{d}X}{\mathrm{d}t}&=-d(t)X(t)+r(t)-F(t,t)\\&=-d(t)X(t)+r(t)-\mathrm{e}^{-\int_{t-\sigma}^{t}d(u)\,\mathrm{d}u}r(t-\sigma).\end{aligned}\tag{B.1}$$

Note that the discounting factor $\mathrm{e}^{-\int_{t-\sigma}^{t}d(u)\,\mathrm{d}u}$ takes the form of an exponential decay with varying decay rate.

In the case of Eqs. (3.10) and (3.11), we have $X=O_K^L$, $r(t)=k\mathcal{J}(p_{\mathrm{TC/B}}\phi(y^L),B_{ij}^L)N_H^L$, and $d(t)=d_{NK}+p_{\mathrm{PK/x}}k\phi(y^L)kx^L$, which yields the discounting factor in (3.12).

Appendix C. The initial concentrations

Assuming that before time 0, no immune reactions have been happening, the initial concentrations of naïve T cells in the lymph node are straightforwardly

$$N_{H,i}^L(t)=s_H/d_{NH},\quad T_{CD8n,i}^L(t)=s_K/d_{NK},\quad T_L^{regn,i}(t)$$
$$i(t)=s_R/d_{regn}$$

for $i=1,2$ and $t\leqslant 0$. The initial concentrations of all other T cells are 0. Likewise, the initial concentration of self-population in the tissue is $T_1^T(t)=s_{T,1}/d_{T,1}$ for $t\leqslant 0$. We assume the foreign population is introduced into the system at time 0, and hence set $T_2^T(t)=0$ for $t<0$ and $T_1^T(0)=1$. The initial value for the discounting factor in (3.11) is $D(0)=\exp(-d_{NK}*\sigma)$.

The total concentrations of immature and mature APCs in the lymph node and tissue depend on the supply, flow, death, and maturation rates of these cells. We assume that APCs were maturing at the non-infectious rate $r_{s,0}$ before time 0, so we calculate the initial total concentrations of immature APCs in the lymph node, immature APCs in the tissue, mature APCs in the tissue, and mature APCs in the lymph node respectively as follows:

$$A_{total}^L(t)=\frac{s_A}{\tilde{f}+d_A+r_{s,0}},$$
$$A_{total}^T(t)=\frac{\tilde{f}A_{total}^L(0)/V}{d_A+r_{s,0}},$$

$$B^T_{total}(t) = \frac{r_{s,0}A^T_{total}(0)}{f/V + d_A},$$

$$B^L_{total}(t) = \frac{r_{s,0}A^L_{total}(0) + fB^T_{total}(0)}{d_A}.$$

The initial concentrations of self antigen in the tissue and lymph node are determined by the supply of antigen by self-population and by the rates of drainage into the lymph node and consumption by APCs. The expression for the initial concentrations turns out to be

$$\alpha_1(t) = \frac{s_\alpha d_{T,1} T_1(0) f}{V + d_\alpha + kA^T_{total}(0)},$$

$$\alpha_1(t) = \frac{f\alpha_1(0) + s_\alpha d_{T,1} T^L_1(0)}{d_\alpha + kA^L_{total}(0)}$$

for $t \leqslant 0$. Since we assume foreign population doesn't appear in the system until time 0, we set the initial concentrations of foreign antigen to be 0.

Table D1
Parameters

Parameters	Description	Figs. 9–20
k	Kinetic coefficient	40
V	Tissue/LN volume ratio	6
$\tilde{f}$	T cell and immature APC outflow rate	0.001
f	Mature APC and antigen inflow rate	5.5
s_H	Naïve CD4 supply rate	8.55
s_K	Naïve CD8 supply rate	6
s_A	APC supply rate	0.48
s_R	Naïve T_{reg} supply rate	0.45
d_{NH}	Naïve CD4 death rate	0.03
d_{NK}	Naïve CD8 death rate	0.03
d_A	APC death rate	0.03
d_{PH}	Primed CD4 death rate	0.2
d_{SH}	Suppressed CD4 death rate	0.2
d_{PK}	Primed CD8 death rate	0.4
d_{SK}	Suppressed CD8 death rate	0.4
d_{CD8nd}	Non-proliferating CD8 death rate	0.4

Table D2
Parameters

Parameters	Description	Figs. 9–20
s_α	Antigen release rate by target	100
$s_{x,1}$	+ signal secretion rate by CD4s	100
$s_{x,2}$	+ signal secretion rate by CD8s	10
s_y	– signal secretion rate by Tregs	100
d_α	Antigen decay rate	5.6
d_g	Growth signal decay rate	5.6
c	Effect of negative signal	1
r_r	Rate CD8s stop proliferating	0.7
r_u	Rate T cells unsuppress	0.7
$s_{T,1}$	Self-population supply rate	0.1
$s_{T,2}$	Foreign-population supply rate	0
$g_{T,1}$	Self-population growth rate	0
$g_{T,2}$	Foreign-population growth rate	0.3
$d_{T,1}$	Self-population death rate	0.01
$d_{T,2}$	Foreign-population death rate	0.1

We calculate the initial concentrations for the separate APC populations by solving the four linear systems

$$dA_{00}/dt = 0,$$
$$dA_{10}/dt = 0,$$
$$dA_{01}/dt = 0,$$
$$A_{00} + A_{10} + A_{01} + A_{11} = A_{total}$$

for immature and mature APCs in the lymph node and tissue.

Appendix D

Table of parameter groups (Tables D1–D4) are given in this section.

Table D3
Parameters

Parameters	Description	Figs. 9–20
n_{NH}	Initial number of CD4 divisions	1
n_{PH}	Further CD4 divisions	1
n_{OK}	Initial number of CD8 divisions	8
n_{PK}	Further CD8 divisions	2
δ_A	Antigen turnover on immature APCs	20
δ_{Am}	Antigen turnover on mature APCs	3
$r_{s,0}$	Normal APC maturation rate	0.01
r_s	Maturation rate during infection	1
$p_{PK/x}$	CD8 response to + signal	0.5
$p_{A/\alpha}$	Antigen incorporation by APCs	0.5
$p_{TC/A}$	Prob of tolerizing reactions for CD4s and CD8s	0.1
$p_{TC/R}$	Prob of T cell suppression	0.5
τ	Duration of T cell division	0.6
σ	Max time in pre-primed CD8 state	1

Table D4
Varied parameters

Parameters	Description	9	10	11	12	13–17	18	20
Anti-self effector reactivities		*Upper-left matrix element*						
$p_{TC/B}$	Naïve CD4/ Mature APC	0.01	0.01	0.01	0.01	0.01	[0,1]	[0,0.1]
$p_{TC/B}$	Primed CD4/ Mature APC	0.01	0.01	0.01	0.01	0.01	[0,1]	[0,0.1]
$p_{TC/B}$	Naïve CD8/ Mature APC	0.01	0.01	0.01	0.01	0.01	[0,1]	[0,0.1]
$p_{A/PH}$	Immature APC/ Primed CD4	0.01	0.01	0.01	0.01	0.01	[0,1]	[0,0.1]
$p_{PK/T}$	Primed CD8/ Target	0.01	0.01	0.01	0.01	0.01	[0,1]	[0,0.1]
Anti-foreign effector reactivities		*Lower-right matrix element*						
$p_{TC/B}$	Naïve CD4/ Mature APC	0.5	0.5	0.5	0.5	0.5	[0,1]	[0,1]
$p_{TC/B}$	Primed CD4/ Mature APC	0.5	0.5	0.5	0.5	0.5	[0,1]	[0,1]
$p_{TC/B}$	Naïve CD8/ Mature APC	0.5	0.5	0.5	0.5	0.5	[0,1]	[0,1]
$p_{A/PH}$	Immature APC/ Primed CD4	0.5	0.5	0.5	0.5	0.5	[0,1]	[0,1]
$p_{PK/T}$	Primed CD8/ Target	0.5	0.5	0.5	0.5	0.5	[0,1]	[0,1]

Table D4 (*continued*)

Parameters	Description	9	10	11	12	13–17	18	20
Anti-self regulatory reactivities	*Upper-left matrix element*							
$p_{R/B}$	Naïve T_{reg}/ Mature APC	0.3	0.3	0.3	0.8	0.6	[0,1]	[0,1]
$p_{R/B}$	Primed T_{reg}/ Mature APC	0.3	0.3	0.3	0.8	0.6	[0,1]	[0,1]
$p_{B/R}$	Mature APC/ Primed T_{reg}	0.3	0.3	0.3	0.8	0.6	[0,1]	[0,1]
Anti-foreign regulatory reactivities	*Lower-right matrix element*							
$p_{R/B}$	Naïve T_{reg}/ Mature APC	0.4	0	0.3	0	0	[0,1]	[0,1]
$p_{R/B}$	Primed T_{reg}/ Mature APC	0.4	0	0.3	0	0	[0,1]	[0,1]
$p_{B/R}$	Mature APC/ Primed T_{reg}	0.4	0	0.3	0	0	[0,1]	[0,1]
All cross-reactivities		0	0	0	0	0	0	0

Appendix E

Table of variable names (Table E1) is given in this section.

Table E1
Names for population variables

Name	Description
T^L	Target concentration in the lymph node (LN)
T^T	Target concentration in tissue
α^L	Antigen concentration in LN
α^T	Antigen concentration in tissue
N_H^L	Naïve CD4+ (helper) T cell concentration in LN
P_H^L	Primed CD4+ (helper) T cell concentration in LN
P_H^T	Primed CD4+ (helper) T cell concentration in Tissue
S_H^L	Suppressed CD4+ (helper) T cell concentration in LN
S_H^T	Suppressed CD4+ T cell concentration in Tissue
N_K^L	Naïve CD8+ (killer) T cell concentration in LN
O_K^L	Pre-primed CD8+ (killer) T cell concentration in LN
P_K^L	Primed CD8+ (killer) T cell concentration in LN
P_K^T	Primed CD8+ (killer) T cell concentration in Tissue
S_K^L	Suppressed CD8+ (killer) T cell concentration in LN
S_K^T	Suppressed CD8+ (killer) T cell concentration in Tissue
Q_K^L	Non-dividing CD8+ (killer) T cell concentration in LN
Q_K^T	Non-dividing CD8+ (killer) T cell concentration in Tissue
A^L	Immature APC concentration in LN
A^T	Immature APC concentration in Tissue
B^l	Mature APC concentration in LN
B^t	Mature APC concentration in Tissue
x^l	Positive signal concentration in LN
x^t	Positive signal concentration in Tissue
y^l	Negative signal concentration in LN
y^t	Negative signal concentration in Tissue
N_R^l	Naïve regulatory T cell concentration in LN
P_R^l	Primed regulatory T cell concentration in LN
P_R^t	Primed regulatory T cell concentration in Tissue
D	Discounting factor for pre-primed CD8+ T cells

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