CHAPTER FOUR

Modeling and Simulation of the Immune System as a Self-Regulating Network

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Contents

Abstract

Numerous aspects of the immune system operate on the basis of complex regulatory networks that are amenable to mathematical and computational modeling. Several modeling frameworks have recently been applied to simulating the immune system, including systems of ordinary differential equations, delay differential equations, partial differential equations, agent-based models,

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and stochastic differential equations. In this chapter, we summarize several recent examples of work that has been done in immune modeling and discuss two specific examples of models based on DDEs that can be used to understand the dynamics of T cell regulation.

1. Introduction

The immune system plays a vital role in human health, with more than 15% of genes in the human genome being linked to immune function (Hackett *et al.*, 2007). The immune system is generally thought to protect against external invaders, such as bacteria, viruses, and other pathogens, while ignoring self. The mechanisms by which the immune system discriminates between self and nonself are becoming elucidated, but are far from being completely understood. Lymphocytes (T and B cells) express antigen receptors generated via novel combinations of gene (V, D, J) segments. This creates an extraordinarily diverse repertoire of unique antigen receptors ($>10^7$ T cell receptors, TCR, in T cells (Arstila *et al.*, 1999); $>10^8$ immunoglobulins, Ig, in B cells (Rajewsky, 1996)) that can respond to potentially all pathogens. However, self-reactive lymphocytes that are also generated in the process could cause autoimmunity if left unchecked. Newly generated T cells mature within the thymus: $\sim$95% die during this process, due to strong binding to self antigens (negative selection) or lack of sufficient signaling (positive selection). Thymic selection is a powerful force that shapes the mature T cell repertoire; this process is referred to as central tolerance. It is now known that potentially autoreactive T cells still persist after thymic selection, so other mechanisms must be operative to keep these in check to maintain peripheral tolerance. A major area of focus in immunology in recent years is regulatory T cells (Tregs), which suppress other immune cells and play an important role in peripheral self tolerance. While there is no organ equivalent to the thymus for B cells, two early tolerance checkpoints regulate developing autoreactive human B cells: the first one at the immature B cell stage in the bone marrow, and the second one at the transition from new emigrant to mature naive B cells in the periphery (Meffre and Wardemann, 2008). As the thymus involutes by young adulthood, how potentially autoreactive T cells are deleted from then on is unclear. New experimental and modeling work suggest that the thymus may play a less prominent role than generally thought in the development of the peripheral T cell pool, even in persons below age 20 (Bains *et al.*, 2009).

While the self/nonself view of immunology makes sense and holds true by and large, exceptions exist upon closer inspection. Since cancer cells are of self origin, it was assumed for decades that the immune system ignores cancer. Yet, approximately 80% of human tumors are infiltrated by T cells,

which appear to have beneficial effects (Galon *et al.*, 2006; Nelson, 2008). Tumor-infiltrating lymphocytes (TILs) have been expanded *in vitro* and their targets have been identified. Contrary to initial expectations, most tumor-infiltrating T cells were found to be directed against self, nonmutated antigens. Such antigens are commonly referred to as tumor-associated antigens or TAAs. Many of the TAAs identified thus far have been in the setting of melanoma (Kawakami and Rosenberg, 1997; Rosenberg, 2001)—the most common ones include MART (melanoma antigen recognized by T cells), gp100, and tyrosinase; others include MAGE, BAGE, GAGE, and NYESO. TAAs have also been identified for breast cancer (e.g., HER-2/neu (Sotiropoulou *et al.*, 2003), MUC (Böhm *et al.*, 1998)), leukemia (e.g., proteinase 3 (Molldrem *et al.*, 1999), WT1 (Oka *et al.*, 2000)), and colon cancer (e.g., CEA (Fong *et al.*, 2001)). Hence, tumor immunity is a form of autoimmunity (Pardoll, 1999). How TAAs which are self, nonmutated proteins break tolerance in the setting of cancer remains poorly understood. This adds complexity to the puzzle of immune regulation.

During a typical infection, the immune response unfolds in multiple waves. The cascade begins with almost immediate responses by innate immune cells, such as neutrophils, which create an inflammatory microenvironment that subsequently attracts dendritic cells and lymphocytes to initiate the adaptive immune response. Perhaps one reason the immune system operates in a series of successive waves rather than in one continuous, concentrated surge is that each burst of immune cells has to be tightly regulated, since some primed cells could potentially give rise to an uncontrolled autoimmune response. Most immune cells exist in different states (resting/active, immature/mature, naïve/effector/memory), which provide additional regulatory mechanisms. What controls the magnitude and duration of each individual response, how does one response give way to another, and induce cellular state changes? More generally, how does the immune system work as such a multifaceted, yet robustly controlled network? In this chapter, we show how principles from mathematical modeling can shed light into understanding how the immune system functions as a self-regulating network. The complexity of the immune system, with emergent properties and nonlinear dynamics, makes it amenable to computational methods for analysis.

1.1. Complexity of immune regulation

As knowledge of the immune system grows it becomes increasingly clear that most immune "decisions" (e.g., whether to attack or tolerate a certain target, or whether to magnify or suppress an immune response) are not made autonomously by individual cells or even by a few isolated cells. Instead, most immune responses result from a multitude of interactions

among various types of cells, continually signaling to one another via cell contact and cytokine-mediated mechanisms.

For example, various types of T cells interact to drive cytotoxic T cell expansion and produce an overall immune response. To begin, T cells must be activated in the lymph node by antigen-presenting cells (APCs), primarily dendritic cells, that present stimulatory or suppressive signals depending on what signals they received while interacting with other cells and cytokines in the surrounding tissue. In the event of infection, APCs usually start by stimulating CD4+ T cells, which begin to multiply and secrete IL-2 and other growth signals that lead to increased activity in the lymph node. Shortly afterward, the cytotoxic (CD8+) T cells get stimulated and begin to proliferate rapidly. Cytotoxic T cells also produce a small amount of IL-2, but mostly direct their energy to extensive proliferation.

Even then, T cell activation follows a more multifaceted route than that already described, for upon stimulation, helper (CD4+) T cells commit to one of two maturation pathways, T_h1 and T_h2, depending on the type of stimulation by APCs and cytokine signals. These pathways direct the adaptive immune response toward cellular or humoral immunity, the first of which is mediated by T cells and macrophages and the latter by B cells and antibodies. Furthermore, in a coregulating network, these separate responses serve to promote their own advancement while suppressing the other. Specifically, activated T_h1 cells release IFN-γ, which promote T_h1 differentiation while hindering T_h2 production, and conversely, activated T_h2 cells release IL-4 and IL-10, which promote T_h2 production while hindering T_h1 cells. Even from this simplified perspective, CD4+ T cell differentiation is governed by a regulatory network, composed of two negatively coupled positive feedback loops.

Another type of T cell associated with the CD4+ family is the regulatory T cell (Treg). As far as currently known, these cells function as a global, negative feedback mechanism that suppresses all activated T cells, down-regulates the stimulatory capacity of APCs, and secretes immunosuppressive cytokines. These cells either emerge directly from the thymus with regulatory capability and are called naturally occurring regulatory T cells (nTregs), or differentiate from nonregulatory T cells after activation and are called antigen-induced regulatory T cells (iTregs). The precise mechanisms governing Treg-mediated regulation are not well understood, although clear evidence shows that Tregs play an essential role in maintaining self tolerance and immune homeostasis (Sakaguchi *et al.*, 1995, 2008). For example, Tregs influence the extent of memory T cell expansion following an immune response via an IL-2-dependent mechanism (Murakami *et al.*, 1998). Furthermore, Tregs may control the extent of effector T cell proliferation during an acute immune response via an IL-2-dependent feedback mechanism (Sakaguchi *et al.*, 2008).

Shifting to another aspect of the adaptive immune response, B cells can be activated by T_h2 cells as discussed above, but they can also respond to antigen without T cell intervention. Many antigens, especially those with repeating carbohydrate epitopes such as those that come from bacteria, can stimulate B cells without T cell intervention. Furthermore macrophages can also display repeated patterns of the same antigen in a way that instigates B cell activation. Yet, most antigens are T cell-dependent, and B cells usually require T cell interaction to achieve maximum stimulation. Nonetheless, not only do T cell-independent mechanisms for B cell activation exist, but experimental evidence shows that B cells also play a role in regulating T cell responses. In particular, the balance between IgG and IgM antibodies secreted by B cells directs the immune response either toward monocytic cells which favor T_h1 production or toward further B cell activity which favor T_h2 production (Bheekha Escura *et al.*, 1995). In later work, Casadevall and Pirofski propose that IgM and IgG may even direct the course of the T cell response by playing proinflammatory and anti-inflammatory roles (Casadevall and Pirofski, 2003, 2006). Hence, T cell/B cell interactions are not unequivocally unidirectional, since stimulatory and suppressive mechanisms operate in a feedback loop through which each cell subpopulation reciprocally influences the other. Furthermore, B cells also exhibit a high level of self-regulation, since antigen-specific antibody responses can be amplified or reduced by several hundredfold via an antibody-mediated feedback mechanism (Heyman, 2000, 2003).

Although this summary only touches a small part of possible immune behavior, it is clear from the myriad interactions among diverse immune cells that nearly all responses are regulated by a huge network of positive and negative feedback loops that consistently keep the global system in check.

1.2. Self/nonself discrimination as a regulatory phenomenon

Another critical aspect of the immune system is self/nonself discrimination. This term refers to the capacity of the immune system to decide whether a particular target is a virulent pathogen, a harmless foreign body, or a normal and healthy self cell. The ensuing immune response must adjust drastically based on the verdict of this decision. Discrimination between target types is largely antigen-based. Certain pathogens, such as bacteria, microbes, and parasites, present protein and carbohydrate sequences that never appear on normal tissue, conspicuously marking them as nonself. The immune system must continually learn over time to recognize certain peptide sequences as normal, while continuing to recognize other sequences as foreign.

Until recently, the prevailing view was that antigen recognition worked by a lock and key mechanism in which adaptive immune cells expressed specific antigen receptors that only responded to one or a few peptide sequences, making it straightforward to see how the immune system

could avoid autoimmunity by removing any immune cells that had a chance of reacting with self antigen. The distinction between self and nonself antigen became blurred, however, when experimental studies revealed that the mature repertoire still maintains self-reactive immune cells; mice depleted of naturally occurring Tregs invariably develop autoimmune disease (Sakaguchi *et al.*, 1995). Furthermore, experimental and quantitative results showed that a high level of cross-reactivity is a central feature of the T cell repertoire (Mason, 1998). These results indicated that T cells react to a range of peptide sequences and that a T cell that primarily reacts to foreign antigen could also potentially cross-react with some self-antigen, thus giving rise to an autoimmune, bystander response against healthy cells.

Due to the intrinsic cross-reactivity of antigen receptors and the inevitable presence of self-reactive immune cells, successful self/nonself discrimination cannot occur as the result of a simple black and white mechanism operating at the individual immune cell level. Instead, this process must emerge from a self-regulatory immune network. Along this line, a novel view of self/nonself discrimination is emerging as a group phenomenon resulting from interactions among several immune agents, including APCs, effector T cells, Tregs, and their molecular signals (Kim *et al.*, 2007).

2. Mathematical Modeling of the Immune Network

As mentioned above, the immune system operates according to a diverse, interconnected network of interactions, and the complexity of the network makes it difficult to understand experimentally. On one hand, *in vitro* experiments that examine a few or several cell types at a time often provide useful information about isolated immune interactions. However, these experiments also separate immune cells from the natural context of a larger biological network, potentially leading to nonphysiological behavior. On the other hand, *in vivo* experiments observe phenomena in a physiological context, but are usually incapable of resolving the contributions of individual regulatory components. To provide a particular example of this shortcoming, our understanding of Treg-mediated regulation and its effect on the immune response is still very poor, even though the majority of individual Treg interactions have already been thoroughly described. This problem of connecting complex, global phenomena to basic interactions extends over a wide range of immunological questions.

How, then, can we take individual-scale results that have been established and connect them to large-scale phenomena? This gap in immunological knowledge provides a fruitful ground for mathematical modeling and computational science. In the following sections, we provide specific

examples of what approaches from mathematical modeling have been applied and what insights have been gained. Table 4.1 gives a summary of advantages, disadvantages, and examples for each modeling approach.

2.1. Ordinary differential equations

Mathematical models based on systems of ordinary differential equations (ODEs) are the most common as these types of models have been used for cancer immunology (de Pillis *et al.*, 2005; Moore and Li, 2004), natural killer cell responses (Merrill, 1981), B cell responses (Lee *et al.*, 2009; Shahaf *et al.*, 2005), B cell memory (De Boer and Perelson, 1990; De Boer *et al.*, 1990; Varela and Stewart, 1990; Weisbuch *et al.*, 1990), Treg dynamics (Burroughs *et al.*, 2006; Carneiro *et al.*, 2005; Fouchet and Regoes, 2008; León *et al.*, 2003, 2004, 2007a,b), and T cell responses (Antia *et al.*, 2003; Wodarz and Thomsen, 2005 to name a few examples.

The primary advantage of ODE modeling is that this model structure has already been extensively applied in the study of reaction kinetics and other physical phenomena. In addition, the mathematical analysis of these systems is relatively simple compared to other types of models and their solutions can be computationally simulated with great efficiency. That is to say, these models can be made extremely complex, before becoming computationally unfeasible.

For example, Merrill constructs an ODE model of NK cell dynamics (Merrill, 1981). In his model, NK cells represent an immune surveillance population that responds immediately to stimulation without the need of prior activation or proliferation. Using this model, he discusses how the NK population could trigger a subsequent T cell response, if necessary, by releasing stimulatory cytokines, such as IFN-γ.

In a model focusing on a different aspect of the immune network, Fouchet and Regoes consider interactions between T cells and APCs to explain self/nonself discrimination (Fouchet and Regoes, 2008). In their model, precursor T cells differentiate into either effector or regulatory T cells depending on whether the stimulation from the APC is immunogenic or tolerogenic. The differentiated effector and regulatory T cells then turn around and drive other APCs to become immunogenic or tolerogenic in two competing positive feedback loops. Furthermore, Tregs also suppress effector T cells. Using this model, Fouchet and Regoes demonstrate how this feedback network causes the immune response to commit to either a fully immunogenic or a fully tolerogenic response, depending on the initial concentration, growth rate, and strength of antigenic stimulus of the target. They also consider how perturbations in the target population may lead to switches between the two network states.

Modeling the adaptive immune system as a whole, Lee *et al.* construct a comprehensive ODE model incorporating APCs, CD4+ T cells, CD8+

Table 4.1 Advantages, disadvantages, and examples of each modeling approach: ODEs, DDEs, PDEs, SDEs, and ABMs

Modeling approach	Advantages	Disadvantages	Examples
ODE	Computationally efficient, describes complex systems elegantly, simple mathematical analysis easy to formulate	Does not capture spatial dynamics or stochastic effects	de Pillis *et al.* (2005), Moore and Li (2004), Merrill (1981), Lee *et al.* (2009), Shahaf *et al.* (2005), De Boer *et al.* (1990), De Boer and Perelson (1990), Varela and Stewart (1990), Weisbuch *et al.* (1990), Burroughs *et al.* (2006), Carneiro *et al.* (2005), Fouchet and Regoes (2008), León *et al.* (2004), León *et al.* (2003), León *et al.* (2007a,b)
DDE	Captures delayed feedback, computationally efficient	Does not capture spatial dynamics or stochastic effects	Kim *et al.* (2007), Colijn and Mackey (2005)
PDE	Captures spatial dynamics and age-based behavior	Computationally demanding, complex mathematical analysis	Antia *et al.* (2003), Onsum and Rao (2007)
SDE	Captures stochastic effects	Computationally demanding, difficult to analyze mathematically	Figge (2009)
ABM	Captures spatial dynamics and individual diversity, captures stochastic effects, easy to formulate	Highly computationally demanding, difficult to analyze mathematically	Catron *et al.* (2004), Scherer *et al.* (2006), Figge *et al.* (2008), Casal *et al.* (2005)

T cells, B cells, antibodies, and two immune environments, lungs and lymph nodes (Lee *et al.*, 2009). Using their model, Lee *et al.* investigate multiple scenarios of infection by influenza A virus, and study the effects of immune population levels, functionality of immune cells, and the duration of infection on the overall immune response. They propose that antiviral therapy reduces viral spread most effectively when administered within two days of exposure. Their highly intricate, multifaceted model demonstrates the ability of mathematical techniques to capture a multitude of dynamic interactions over a broad spectrum of cell types.

2.2. Delay differential equations

Systems of ODEs are finite dimensional dynamical systems, while delay differential equations (DDEs) and partial differential equations (PDEs) are infinite-dimensional dynamical systems. As a result, DDEs and PDEs require more computational and analytical complexity than their finite dimensional counterparts. However, infinite-dimensional systems come with unique modeling advantages.

In general, DDEs are simpler than PDEs. DDE models are also similar in structure to ODE models, except that they explicitly include time delays. Many biological processes exhibit delayed responses to stimuli, and DDE models allow us to understand the effects of these delays on a feedback network.

An example of a model that makes use of DDEs is the work by Colijn and Mackey (2005) in which they model the development of neutrophils from stem cells (i.e., neutrophil hematopoiesis). Neutrophils that have attained maturity release a molecular signal that causes cells earlier in development to stop differentiating. Ideally, this signaling gives rise to a delayed negative feedback that ultimately stabilizes the neutrophil population at an equilibrium. However, the long delay in the signal permits a situation in which the neutrophil population never stabilizes, but continues to oscillate from unusually high to unusually low levels. Colijn and Mackey connect this oscillatory dynamic to cyclical neutropenia, a disease that causes patients to have periodically low levels of neutrophils.

Another example is our recent work in which we devise a mathematical model to study the regulation of the T cell response by naturally occurring Tregs (Kim *et al.*, 2007). In this model, we consider a variety of immune agents, including APCs, CD4+ T cells, CD8+ T cells, Tregs, target cells, antigen, and positive and negative growth signals. Furthermore, each immune population can migrate between two distinct environments: the lymph node and the tissue. We also consider various time scales, such as a long delay between initial CD8+ T cell stimulation and full activation and a much shorter delay for each T cell division. The delays cause the CD8+ response to initiate with a time lag after the CD4+ response. In addition,

the delay due to cell division ensures that the Treg response develops more slowly than the other two T cell responses, allowing a small time window of unrestricted T cell expansion.

The delays produce another unexpected phenomenon, a two-phase cycle of T cell maturation. In the first phase, CD4+ T cells expand and secrete positive growth signal allowing CD8+ T cells to proliferate rapidly, whereas in the second phase, the Treg population catches up to the original effector T cell population and begins suppressing T cell activity, causing a sudden shift from proliferation to emigration from the lymph node into the peripheral tissue, where CD8+ T cells can more effectively eliminate the target population.

From a practical point of view, DDE models are only slightly more complex than ODE models to simulate numerically. Evaluating DDE systems reduces to recording the past history of all populations throughout the simulation. Hence, with only a slight increase in computational complexity, DDE models widely expand the repertoire of phenomena that can be attained.

2.3. Partial differential equations

PDE models capture more complexity than DDE and ODE models. In biological modeling, PDEs are often applied in two ways, age-structured and spatio-temporal models.

Age-structured models account for the progression of individual cells or members through a scheduled development process. As many organisms exhibit behaviors that depend on their maturity and developmental level, age-structured models provide a useful framework for modeling internal development of an organism over time.

For example, Antia *et al.* formulate an age-structured model to simulate the progression of cytotoxic T cells through an autonomous T cell proliferation program (Antia *et al.*, 2003). According to the program, activated T cells enter into a scheduled period of expansion, and then relative stabilization, followed by a period of contraction, and then restabilization at a lower level. These four stages of scheduled development comprise the T cell proliferation program. Using this model, they study the effect of variations in the T cell program on the level and duration of cytotoxic T cell responses. Furthermore, they conclude that T cell responses that are governed by autonomous, intracellular programs will execute similarly despite a wide range of antigen stimulation levels. This latter phenomenon has also been observed experimentally (Kaech and Ahmed, 2001; Mercado *et al.*, 2000; van Stipdonk *et al.*, 2003).

Returning to the notion of regulation, a T cell program such as the one modeled by Antia *et al.* (2003), or any other scheduled developmental process, implies a system of internal self-regulation that may be invisible

to the external network, but that results from diverse interactions within the cell. Due to the inherent difficulty of simultaneously modeling feedback networks on intracellular and extracellular levels, age-structured models provide an efficient tool for investigating the interactions between internal and external regulatory mechanisms.

Another classical and highly useful application of PDE models is modeling spatio-temporal dynamics. Using this approach, Onsum and Rao develop a PDE model for neutrophil migration toward a site of infection by moving toward higher chemical concentrations (Onsum and Rao, 2007). They simulate how two chemical signals interacting in an antagonizing manner allow neutrophils to orient themselves within the chemical gradient. Their PDE model is composed of a system of diffusion and chemotaxis equations in one space dimension.

From the viewpoint of deterministic, differential equations, PDEs provide the most powerful mathematical modeling tool that captures the broadest range of biological phenomena. These models have, however, the potential to be significantly more computationally demanding than ODE and DDE systems.

2.4. Agent-based models

The concept of an agent-based model (ABM) refers to a different modeling philosophy than that used in differential equation systems. First of all, ABMs deal with discrete and distinguishable agents, such as individual cells or isolated molecules, unlike differential equations, which deal with collective populations, such as densities of cells. In addition, ABMs easily allow us to account for probabilistic uncertainty, or stochasticity, in biological interactions. For example, in a stochastic ABM, an individual agent only changes state or location at a certain probability and not by following a deterministic process. Finally, as with PDEs, most ABMs consider the motion of agents through space.

A powerful application of ABMs is demonstrated by Catron *et al.* (2004). They devise a sophisticated ABM to simulate the interaction between a T cell and a dendritic cell in the lymph node. By observing repeated simulations of T cell–DC interactions, they obtain estimates of the frequency of T cell–DC interactions and the expected time for T cells to become fully stimulated.

In another ABM, Scherer *et al.* simulate T cell competition for access to binding sites on mature antigen-bearing APCs (Scherer *et al.*, 2006). As in standard first-order reaction kinetics (i.e., the law of mass action), T cells interact with APCs with a probability proportional to the product of their two populations. Furthermore, each APC possesses a finite number of antigen binding sites that can each present either of the two types of antigen simulated in the model. Using their model, Scherer *et al.* determine that the

nature of T cell competition changes depending on the level of antigen expressed by the APCs. More specifically, under low antigen expression, T cells of the same antigen-specificity are more likely to compete, allowing for the coexistence of multiple T cell responses against different target epitopes. On the other hand, under high antigen expression, T cell competition becomes more indiscriminate, ultimately allowing highly reactive T cell populations to competitively exclude T cell populations that are specific for different epitopes. Using an agent-based approach, this model demonstrates how intercelluar competition can indirectly provide a means of T cell regulation.

At the cellular level, Figge *et al.* simulate B cell migration in the germinal center of a lymph node (Figge *et al.*, 2008). In their model, they assume that individual B cells move according to a random walk attempting to follow a chemoattractant. They apply their model for the purpose of resolving the paradox obtained from two-photon imaging data that B cell migration initially appears follow a chemotactic gradient but then devolves into what resembles more of an undirected random walk. Using their simulations, they hypothesize that chemotaxis must remain active throughout the entire B cell migration process as to maintain a sense of the germinal center. At the same time, individual B cells downregulate chemokine receptors causing them to lose sensitivity to the chemical gradient.

On the molecular level, Casal *et al.* construct an ABM for T cell scanning of the surface of an APC. In this model, the agents are individual T cell receptors and peptide sequences that populate the surfaces of interacting cells (Casal *et al.*, 2005).

The main advantage of agent-based modeling is the ability to account for probabilistic uncertainty and individual diversity within a large population. The main difficulty is, on the other hand, the huge computational complexity that accompanies such sophisticated models. Roughly speaking, most ABMs take on the order of several hours to even days to simulate even once, whereas most deterministic models can be evaluated much faster. Furthermore, stochastic ABMs usually have to be simulated numerous times to obtain the overall average behavior of the system. Thus, despite their advantages ABMs often present great challenges in terms of computational implementation.

2.5. Stochastic differential equations

Perhaps the least explored path in immunological modeling is the use of stochastic differential equations (SDEs). From the point of view of complexity, SDEs lie somewhere in-between deterministic, differential equation models, and ABMs. SDEs are written and formulated a lot like ODEs, except that they allow their variables to take random values. Traditionally, SDEs provide an effective means of accounting for noise, random walks,

and sporadic events (modeled as a Poisson process), and they have been applied extensively in financial mathematics, chemistry, and physics. However, they have not yet fully made an entry into mainstream immunological modeling.

Nonetheless, we can provide one example of an SDE model that has been applied to X-linked agammaglobulinemia, a genetic disorder of B cell maturation that prevents the production of immunoglobulin. In his model, Figge formulates a system of SDEs to simulate the depletion of immunoglobulin by natural degradation and antigenic consumption and its periodic replenishment by immunoglobulin substitution therapy (Figge, 2009). The stochastic model captures the tendency of the immunoglobulin repertoire to shift toward certain antigen-specificities at the expense of others. In addition, the regulatory network clarifies how immunoglobulin substitution therapy may affect other aspects of the overall immune response in ways that were not clear before. Figge's assessment of the current treatment strategy is that lower treatment frequencies, separated by a period of one to several weeks, may actually benefit the prevention of chronic infection.

The computational complexity of SDEs generally falls between that of deterministic models and ABMs. SDE models are one step above ODE models in terms of their complexity, because they incorporate stochastic effects. Nonetheless, like ODEs, SDEs still consider populations as collective groups rather than as individual agents.

2.6. Which modeling approach is appropriate?

Such a diverse selection of available models, not to mention possible hybrid formulations, begs the question, ''Which modeling approach is most appropriate?'' The answer depends on the nature of regulatory interactions involved, among other issues.

As discussed, ODEs are the most efficient method for modeling huge levels of biological complexity without a substantial increase in the computational work. For any regulatory networks that do not rely significantly on delayed feedback, spatial distribution of cells and molecules, or probabilistic events, ODE models are the most effective approach. For networks that seem to depend on delayed feedback, DDEs provide a good paradigm and also remain relatively simple from a computational point of view.

Networks of cells and molecules that do not mix well or efficiently, but remain localized over a long period of time, may correspond most appropriately to PDE models that account for space. Similarly, networks of cells that change behavior gradually over time also lend themselves naturally to age or maturity-structured formulations using PDEs. Moving beyond deterministic models, SDEs provide one means of adding stochasticity to differential equations, but they come with a higher level of computational complexity.

In recent years, there has been a growth in the use of ABMs. The ABM paradigm currently provides the most complex and versatile framework for mathematical modeling by incorporating all elements of spatial and temporal dynamics, probabilistic events, and individual diversity within populations. However, ABMs demand by far the most intensive computational algorithms and are often impractical for statistical analyses such as parameter sensitivity or data fitting, which usually require numerous simulations. As a paradigm, ABMs seek to closely replicate the complexity of biological systems, so that "experiments" can be done *in silico* much more economically and even ethically than they could be performed *in vivo*.

On one hand, ABMs provide practical means of transporting experimental studies from the wet lab to the computer lab. However, ABMs do not replace other forms of modeling because they do not substantially reduce the inherent complexity of biological systems. Furthermore, when applying ABMs to an immunological network, one should confirm that the dynamic behavior of the ABM cannot be sufficiently recreated by a simpler, differential equation formulation. For example, the two papers (Doumic-Jauffret, 2009; Kim *et al.*, 2008) succeed in replicating the dynamics of a highly complex ABM with almost no deviation using two deterministic models: difference equations and PDEs. In addition, these deterministic models require only 4 min and 30 s of computation time as opposed to the approximately $50 \times 7 = 350$ h required by the ABM. This result demonstrates the efficacy of hybrid methods that merge both ABM and differential equation frameworks to capture the underlying characteristics of a biological network without adding any superfluous detail.

In practice, it is difficult to predict which mathematical and computational paradigm is most suitable for a given situation. Ideally, to thoroughly understand a system from a modeling perspective, one should devise mathematical models of all types for each immunological network in question. This line of thinking is, needless to say, unreasonable. Instead, the rational and the most informed approach to mathematical modeling is to recognize the capacities and limitations of each type of model and to apply the paradigm that most accurately quantifies the essential dynamics of the system without introducing any unnecessary complexity.

3. Two Examples of Models to Understand T Cell Regulation

In the following section, we provide two examples of DDE models based on immune regulatory networks that were proposed by Antia *et al.* (2003) and Kim (2009). Each of the models describes a distinct network that could regulate T cell development during an acute infection.

3.1. Intracellular regulation: The T cell program

The first regulatory network is based on the notion of a *T cell proliferation program*. According to this concept, T cells follow a fixed program of development that initiates after stimulation and then proceeds to unfold without any further feedback from the environment. As mentioned in Section 2.3, a programmed cellular response implies an intracellular regulatory mechanism that may still be highly complex, although it no longer interacts with the rest of the external network. Our example comes from Kim (2009), and it stems from the original T cell program model formulated by Antia *et al.* (2003) and further developed by Wodarz and Thomsen (2005). The T cell program (illustrated in Fig. 4.1) can be summarized as follows:

1. APCs mature, present relevant target antigen, and migrate from the site of infection to the draining lymph node.
2. In the lymph node, APCs activate naïve T cells that enter a minimal developmental program of m cell divisions.
3. T cells that have completed the minimal developmental program become effector cells that can divide in an antigen-dependent manner (i.e., upon further interaction with APCs) up to n additional times.
4. Effector cells that divided the maximum number of times stop dividing.

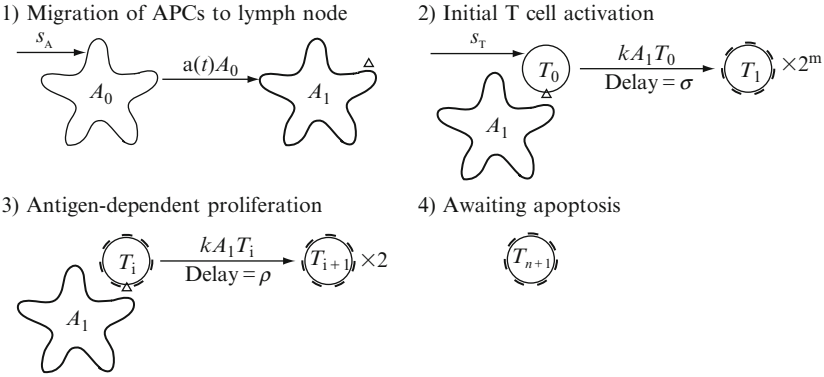


Figure 4.1 The T cell program. (1) Immature APCs pick up antigen at the site of infection at a time-dependent rate $a(t)$. These APCs mature and migrate to the lymph node. (2) Mature antigen-bearing APCs present antigen to naïve T cells causing them to activate and enter the minimal developmental program of m divisions. (3) Activated T cells that have completed the minimal program continue to divide upon further interaction with mature APCs for up to n additional divisions. (4) T cells that have completed the maximal number of divisions stop dividing and wait for apoptosis. Although not indicated, each cell in the diagram has a natural death rate according to its kind.

This process can be translated into a system of DDEs in which each equation corresponds to one of the cell populations shown in Fig. 4.1. The system of DDEs is as follows:

$$A_0'(t) = s_A - d_0 A_0(t) - a(t) A_0(t), \tag{4.1}$$

$$A_1'(t) = a(t) A_0(t) - d_1 A_1(t), \tag{4.2}$$

$$T_0'(t) = s_T - \delta_0 T_0(t) - k A_1(t) T_0(t), \tag{4.3}$$

$$T_1'(t) = 2^m k A_1(t-\sigma) T_0(t-\sigma) - k A_1(t) T_1(t) - \delta_1 T_1(t), \tag{4.4}$$

$$T_i'(t) = 2k A_1(t-\rho) T_{i-1}(t-\rho) - k A_1(t) T_i(t) - \delta_1 T_i(t), \tag{4.5}$$

$$T_{n+1}'(t) = 2k A_1(t-\rho) T_n(t-\rho) - \delta_1 T_{n+1}(t). \tag{4.6}$$

The variables in the equations have the following definitions:

- A_0 is the concentration of APCs at the site of infection.
- A_1 is the concentration of APCs that have matured, started to present target antigen, and migrated to the lymph node.
- T_0 is the concentration of antigen-specific naïve T cells in the lymph node.
- T_{i+1} is the concentration of effector cells that undergone i antigen-dependent divisions after the minimal developmental program.
- T_{n+1}, denotes T cells that have undergone n divisions after the minimal developmental program. These cells have terminated the proliferation program and can no longer divide.

Cell concentration is measured in units of k/μL (thousands of cells per microliter).

Figure 4.2 shows an expanded diagram of how the first two equations, Eqs. (4.1) and (4.2), are derived from step 1. Equation (4.1) pertains to the

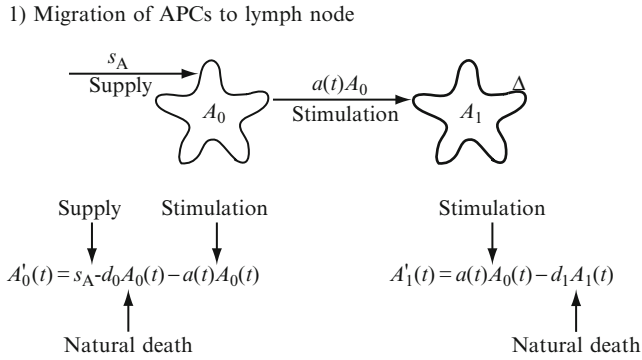


Figure 4.2 Expanded diagram of how Eqs. (4.1) and (4.2) are derived from step 1 of the T cell program.

population of immature APCs waiting at the site of infection. These cells are supplied into the system at a constant rate, s_A, and die at a proportional rate, d_0. Without stimulation, this population always remains at equilibrium, given by s_A/d_0. The time-dependent coefficient $a(t)$ denotes the rate of stimulation of APCs as a function of time. The function $a(t)$ can be seen as being proportional to the antigen concentration at the site of infection.

Equation (4.2) pertains to the population of APCs that have matured, started to present relevant antigen, and migrated to the lymph node. For simplicity, the model accounts for the maturation, presentation of antigen, and migration of APCs as one event. The first term of the equation corresponds to the rate at which these APCs enter the lymph node as APCs at the site of infection are stimulated. The second term is the natural death rate of this population.

Figure 4.3 shows an expanded diagram of how Eqs. (4.3) and (4.4) are derived from step 2. Equation (4.3) pertains to naïve T cells. This population is replenished at a constant rate, s_T, and dies at a proportional rate, δ_0. Without stimulation, the population remains at equilibrium, s_T/δ_0. The third term in this equation is the rate of stimulation of naïve T cells by mature APCs. The bilinear form of this term follows the law of mass action where k is the kinetic coefficient.

Equation (4.4) pertains to newly differentiated effector cells that have just finished the minimal developmental program of m divisions. The first term gives the rate at which activated naïve T cells enter the first effector state, T_1. This term corresponds to the final term of the previous equation for $T_0'(t)$, except that it has an additional coefficient of 2^m and it depends on cell concentrations at time $t-\sigma$. The coefficient 2^m accounts for the increase in population of naïve T cells after m divisions, and the time delay σ is the

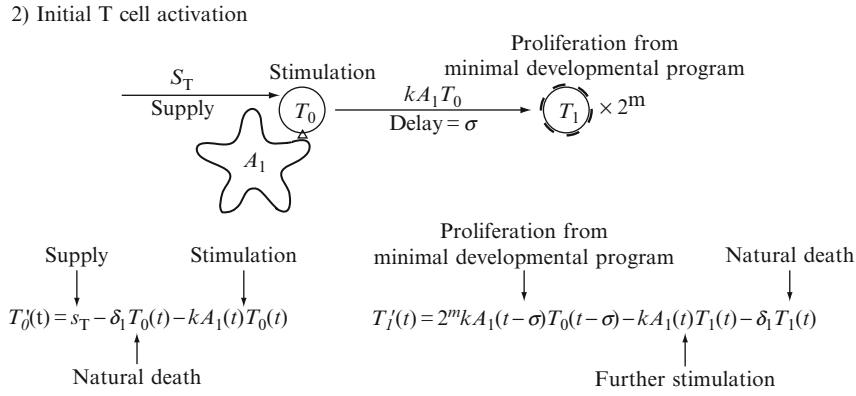


Figure 4.3 Expanded diagram of how Eqs. (4.3) and (4.4) are derived from step 2 of the T cell program.

duration of the minimal developmental program. This term accounts for newly proliferated effector cells that appear in the T_1 population σ time units after activation from T_0. The second term is the rate at which T_1 cells are stimulated by mature APCs for further division. It is based on the law of mass action and is of the same form as the final term of the equation for $T_0'(t)$. This term exists in the equation only if the number of possible antigen-dependent divisions, n, is not 0. Finally, as shown by the last term, T_1 cells continuously die at rate δ_1.

Figure 4.4 shows an expanded diagram of how Eq. (4.5) is derived from step 3 and how Eq. (4.6) is derived from step 4. For $i = 2, \ldots, n$, Eq. (4.5) for $T_i'(t)$ is analogous to the equation for $T_1'(t)$, except that these cells only divide once after stimulation. Hence, the coefficient of the first term is 2, and the time delay is ρ, the duration of a single division. As before, the second term is the rate at which these cells become stimulated for further division, and the final term is the death rate. Note that we use the same death rate, δ_1, for all effector cells.

The final equation, Eq. (4.6), pertains to cells that have undergone the maximum number of possible antigen-dependent divisions. These cells do not divide anymore and can only die at rate δ_1.

The parameter estimates used for this model come from Kim (2009) and are summarized in Table 4.2. The function $a(t)$, representing the rate of antigen stimulation, is defined by

$$a(t) = c\frac{\phi(t)\phi(b-t)}{\phi(b)^2}, \tag{4.7}$$

where

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

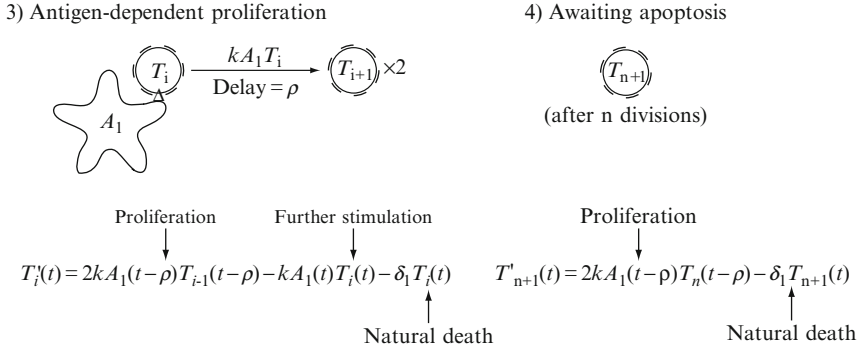


Figure 4.4 Expanded diagram of how Eq. (4.5) is derived from step 3 and how (4.6) is derived from step 4 of the T cell program.

Table 4.2 Estimates for model parameters

Parameter	Description	Estimate
$A_0(0)$	Initial concentration of immature APCs	$s_A/d_0 = 10$
$T_0(0)$	Initial concentration of naïve T cells	$s_T/\delta_0 = 0.04$
s_A	Supply rate of immature APCs	0.3
s_T	Supply rate of naïve T cells	0.0012
d_0	Death/turnover rate of immature APCs	0.03
δ_0	Death/turnover rate of naïve T cells	0.03
d_1	Death/turnover rate of mature APCs	0.8
δ_1	Death/turnover rate of effector T cells	0.4
k	Kinetic coefficient	20
m	Number of divisions in minimal developmental program	7
n	Maximum number of antigen-dependent divisions	3–10
ρ	Duration of one T cell division	1/3
σ	Duration of minimal developmental program	3
$a(t)$	Rate of APC stimulation	Eq. (4.7)
b	Duration of antigen availability	10
c	Level of APC stimulation	1
r	Rate of differentiation of effector cells into iTregs	0.01

Concentrations are in units of k/μL, and time is measured in days.

and b, $c > 0$. This function starts at 0, increases to a positive value for some time, and returns to 0. Kim (2009) demonstrated the duration of antigen availability, b, is estimated to be 10 days, and the level of APC stimulation, c, is estimated to be 1. (See Fig. 4.5 for graphs of $a(t)$ for $b = 3$ and $b = 10$ when $c = 1$.)

3.2. Intercellular regulation: iTreg-based negative feedback

The second regulatory network is based on a negative feedback loop mediated by iTregs that differentiate from effector T cells during the course of the immune response. Several mathematical models considering Treg-mediated feedback have been developed for naturally occurring regulatory T cells (nTregs) (Burroughs *et al.*, 2006; León *et al.*, 2003) and iTregs (Fouchet and Regoes, 2008), but these models focus on the function of Tregs in maintaining immune tolerance. In contrast, the following model

focuses on the primary response against acute infection rather than long-term behavior. The model comes from Kim's study (2009).

In this feedback network, T cell responses begin the same way as for the T cell program. However, T cell contraction initiates differently, since it is mediated by external suppression by iTregs. This process (illustrated in Fig. 4.6) can be described in five steps:

1. APCs mature, present relevant target antigen, and migrate from the site of infection to the draining lymph node.

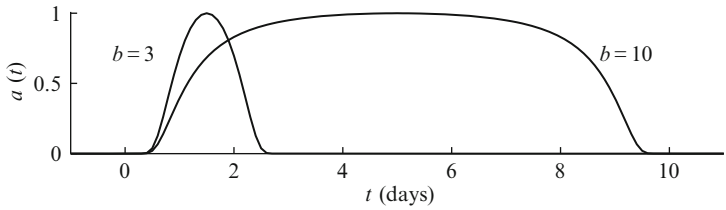
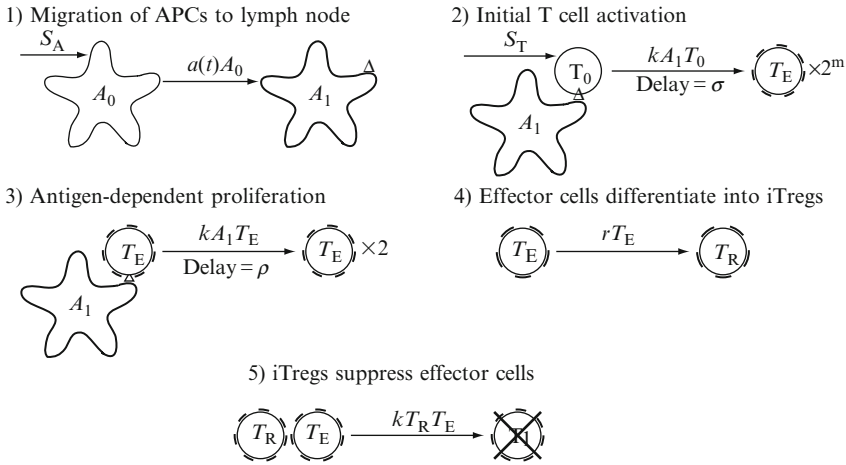


Figure 4.5 Graphs of the antigen function $a(t)$ given by Eq. (4.7) for $b = 3$ and $b = 10$ when $c = 1$. The function $a(t)$ represents the rate that immature APCs pick up antigen and are stimulated.



(although not indicated, each cell has a natural death rate according to its kind.)

Figure 4.6 Diagram of the iTreg model. The first three steps are identical to those in the cell division-based model that is shown in Fig. 4.1. In the fourth step, effector cells differentiate into iTregs at rate r. In the fifth step, iTregs suppress effector cells. Although not indicated, each cell in the diagram has a natural death rate according to its kind.

2. In the lymph node, APCs activate naïve T cells that enter a minimal developmental program of m cell divisions.
3. T cells that have completed the minimal developmental program become effector cells that keep dividing in an antigen-dependent manner as long as they are not suppressed by iTregs.
4. Effector cells differentiate into iTregs at a constant rate.
5. The iTregs suppress effector cells upon interaction.

The model can be formulated as a system of five DDEs shown below:

$$\begin{aligned} A_0'(t) &= s_A - d_0 A_0(t) - a(t) A_0(t), \\ A_1'(t) &= a(t) A_0(t) - d_1 A_1(t), \\ T_0'(t) &= s_T - \delta_0 T_0(t) - k A_1(t) T_0(t), \\ T_E'(t) &= 2^m k A_1(t-\sigma) T_0(t-\sigma) T_0(t-\sigma) \\ &\quad - k A_1(t) T_E(t) + 2k A_1(t-\rho) T_E(t-\rho) \\ &\quad - (\delta_1 + r) T_E(t) - k T_R(t) T_E(t). \end{aligned} \tag{4.8}$$

$$T_R'(t) = r T_E(t) - \delta_1 T_R. \tag{4.9}$$

As in the previous model, A_0 is the concentration of APCs at the site of infection, A_1 is the concentration of APCs that have matured, started to present target antigen, and migrated to the lymph node, and T_0 is the concentration of naïve T cells in the lymph node. In addition, T_E is the concentration of effector cells, and T_R is the concentration of iTregs.

The first three equations for APCs and naïve T cells are identical to those in the T cell program model from Section 3.1. The first two terms of Eq. (4.8) for $T_E'(t)$ are identical to the first two terms of Eq. (4.4) for the T cell program. The third term in this equation is the rate that cells that have just finished dividing reenter the effector cell population. In this model, cells do not have a programmed maximum number of divisions, so it is not necessary to count the number of divisions a cell has undertaken. The only regulatory mechanism is suppression by iTregs. The fourth term is the rate that effector cells exit the population through death at rate δ_1 or differentiate into iTregs at rate r. The final term is the rate that effector cells are suppressed by iTregs. As before, the rate of iTreg–effector interactions follows the same mass action law as APC–T cell interactions.

Equation (4.9) pertains to iTregs. The first term is the rate at which effector cells differentiate into iTregs, and the second term is the rate at which iTregs die. The iTregs have the same death rate as effector cells.

All parameters in this model are identical to those used for the T cell program, except for r, the rate of differentiation of effector cells into iTregs. As Kim (2009) demonstrated, we estimate that $r = 0.01$/day, meaning that 1% of effector cells differentiate into iTregs per day. The parameters used in the iTreg model are listed in Table 4.2.

4. How to Implement Mathematical Models in Computer Simulations

Once a mathematical model has been developed, the next step is to implement it computationally. A common approach is to write the relevant computational software for each problem, since this method has the advantage of allowing the programmer to optimize the computer algorithms for his or her particular needs. However, various software packages already exist for most of the modeling paradigms. For ABM simulations, the immune system simulator (IMMSIM) (Celada and Seiden, 1992; Seiden and Celada, 1992), the synthetic immune system (SIS) (Mata and Cohn, 2007), the basic immune simulator (BIS) (Folcik *et al.*, 2007) provide platforms for generating virtual immune systems populated by a variety of cell types. For deterministic, differential equation models, the most frequently used programs are MATLAB, Maple, and XPPAUT. In general, DDE models are relatively simple to evaluate on any of the software platforms for differential equations mentioned above, and we numerically simulate the DDE models from Sections 3.1 and 3.2 with the "dde23" function of MATLAB R2008a.

Currently, no widely used computational tools exist for evaluating SDE models for biological systems, since this direction of research has yet to be developed. Although numerous programs are available for pricing financial devices, these approaches are usually not ideal for models pertaining to immunological networks.

4.1. Simulation of the T cell program

The following simulations are drawn from Kim's study (2009) and primarily focus on the effect of antigen stimulation levels and precursor concentrations on the magnitude of the T cell response. Before proceeding to numerical simulations, the first crucial point to notice is that the T cell dynamics given by Eqs. (4.1)–(4.6) directly scale with respect to the precursor concentration, $T_0(0)$. In other words, a T cell response that begins with x times as many precursors as another automatically has a peak that is x times higher. This scaling property holds, because T cell program model is linear with respect to the T cell populations, $T_i(0)$. As a result, simulations pertaining to the T cell program only consider relative T cell expansion levels, given by $T_{\text{total}}(t)/T_0(0)$, rather than total T cell populations given by

$$T_{\text{total}}(t) = \sum_{i=1}^{n} \left(T_i(t) + \int_{t-\rho}^{t} kA_1(u)\mathrm{d}u \right) + T_{n+1}(t).$$

Note that Eqs. (4.4)–(4.6) imply that stimulated T cells leave the system during the division process and return ρ time units later. Hence, the total T cell concentration is not only the sum of T cell populations given by $T_i(t)$,

but also the populations that are undergoing division, which are given by the integrals in the above expression.

The first set of simulations examines the dependence of the T cell peak on the two antigen-related parameters, c and b, corresponding to the level and duration of antigen presentation, respectively. We use the parameters listed in Table 4.2, and c and b vary from 0.1 to 3 and from 1 to 15 days, respectively. The maximum T cell expansion level versus c is plotted in Fig. 4.7A, and the maximum T cell expansion level versus b is plotted in 4.7B. To understand the T cell behavior under a variety of possible T cell programs, we use the lowest and highest estimated values (3 and 10) of n, which denotes the maximum possible number of antigen-dependent divisions after the minimal developmental program. We draw the two corresponding curves for each value of n in each plot.

As can be seen in Fig. 4.7A, T cell dynamics saturate very quickly in relation to c, so much so that the doubling level period is almost constant

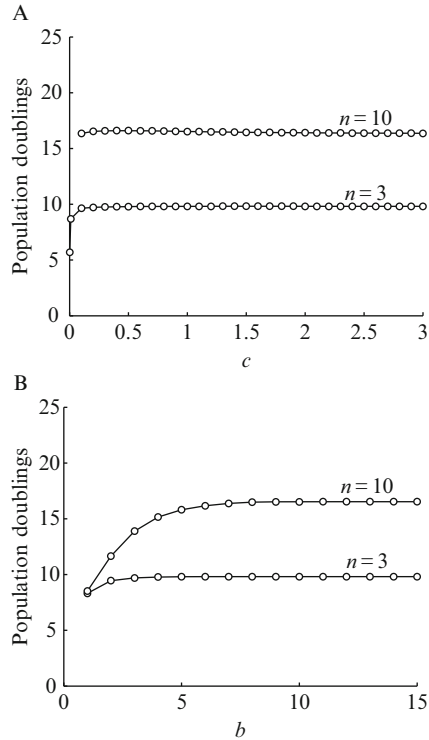


Figure 4.7 Dependence of T cell dynamics on c, a parameter corresponding to the level of antigen presentation, and on b, the duration of antigen availability. (A) Maximum T cell expansion level versus c. Expansion level is measured in population doublings, which is defined by $\log_2(\max(T_{\text{total}}/T_0(0)))$. Data is shown for the two possible values of n, the maximum number of possible antigen-dependent divisions after the minimal developmental program. (B) Maximum T cell expansion level versus b.

from as low as $c = 0.1$ to as high as $c = 3$. By continuity, the size of the T cell peak must go to 0 as c decreases, but the drop is very steep. The two extra points shown in the curves for $n = 3$ correspond to $c = 0.001$ and $c = 0.01$. These values correspond to roughly 0.1% and 1% of APCs getting stimulated per day. Hence, even very low stimulation levels result in nearly saturated T cell dynamics.

The plots of the maximum expansion level and the time of peak versus b are shown in Fig. 4.7B. The figure shows that T cell dynamics also saturate as b increases, but not as quickly as for c. Hence, the simulations show that the duration of antigen availability is more important than the level. The plots on Fig. 4.7B show that for both $n = 3$ and $n = 10$, T cell expansion levels begin to saturate around $b = 4$ or 5 days, indicating that the immune response behaves similarly as long as antigen remains available for long enough to elicit a fully developed T cell response.

As a final simulation for the T cell program model, Fig. 4.8 shows the time evolution of various cell populations when $n = 10$ and the rest of the

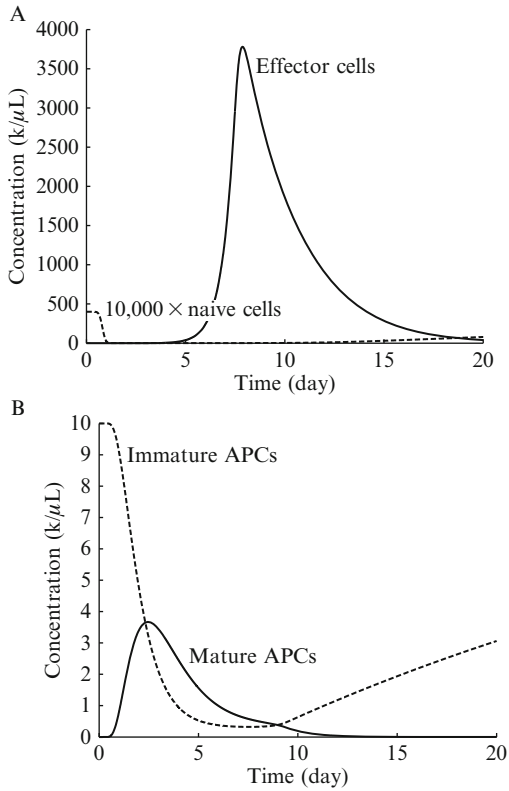


Figure 4.8 Time evolution of immune cell populations over time. (A) The dynamics of naïve and effector cells over 20 days. (B) The dynamics of immature and mature APCs.

parameters are taken from Table 4.2. In Fig. 4.8A, the T cell peak is 94605 times higher than the precursor concentration, $T_0(0) = 0.04$ k/μL. As mentioned at the beginning of this section, the ratio between the T cell peak and the precursor concentration remains constant for all values of $T_0(0)$. Since this relation is exactly linear, we do not give a plot of the maximum height of T cell expansion versus precursor concentration.

4.2. Simulation of the iTreg model

The simulations in this section are also drawn from Kim's study (2009) and follow a similar pattern to those in Section 4.1. Due to the negative feedback from iTregs in Eq. (4.8), T cell dynamics do not directly scale with respect to precursor frequencies as in the T cell program model. In this case, it is informative to look at the total effector cell population, given by

$$T_{\text{total}}(t) = T_1(t) + \int_{t-\rho}^{t} kA_1(u)T_1(u)\mathrm{d}u.$$

Figure 4.9 displays a log–log plot of the maximum expansion level versus the initial naïve T cell concentration, $T_0(0)$, which is varied from 4×10^{-4} to 4 k/μL, a range 100 times lower and 100 times higher than the estimated value in Table 4.2. As shown in Fig.4.9A, the simulated data fits a power law of exponent 0.3004, meaning that the expansion roughly scales to the cubed

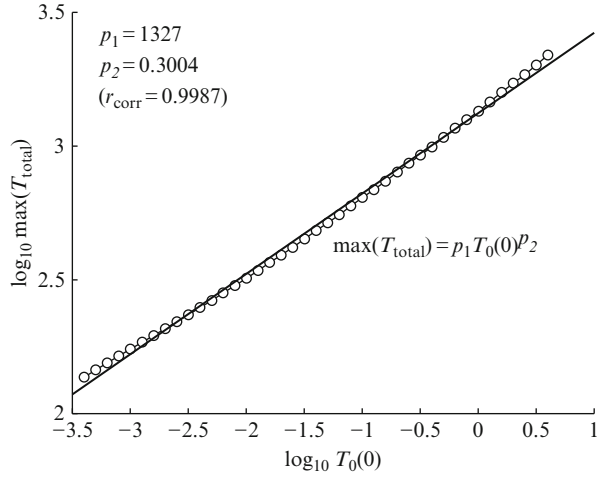


Figure 4.9 A log–log plot of the dependence of the T peak on $T_0(0)$, the initial concentration of naïve T cells. The linear regression shows that the maximum T cell expansion level is roughly proportional to $T_0(0)^{1/3}$. The linear correlation $r_{\text{corr}} = 0.9987$.

root of the initial naïve cell concentration. For example, to obtain a T cell response that is 10 times higher (or lower) than normal, the system would need to start with a reactive precursor concentration that is 1000 times higher (or lower) than normal.

Following the same sensitivity analysis as in Section 3.1, we see in Fig. 4.10 that the dynamics of the iTreg model exhibits similar saturating behavior with respect to the level and to the duration of antigen stimulation, given by c and b, respectively. Like the program-based model, the feedback model generates dynamics that behave insensitively to the level and duration of antigen stimulation.

Figure 4.11 shows the time evolution of the effector and iTreg populations when all other parameters are taken from Table 4.2. The figure

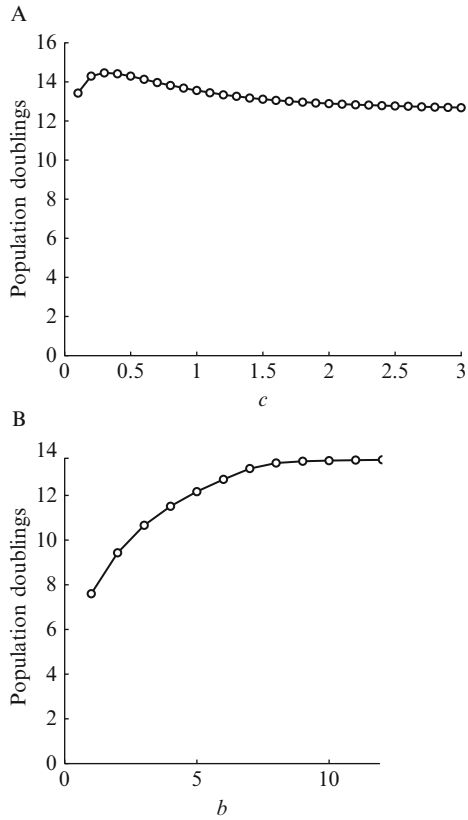


Figure 4.10 Dependence of T cell dynamics on c, a parameter corresponding to the level of antigen presentation, and on b, the duration of antigen availability. (A) Maximum T cell expansion level versus c. (B) Maximum T cell expansion level versus b.

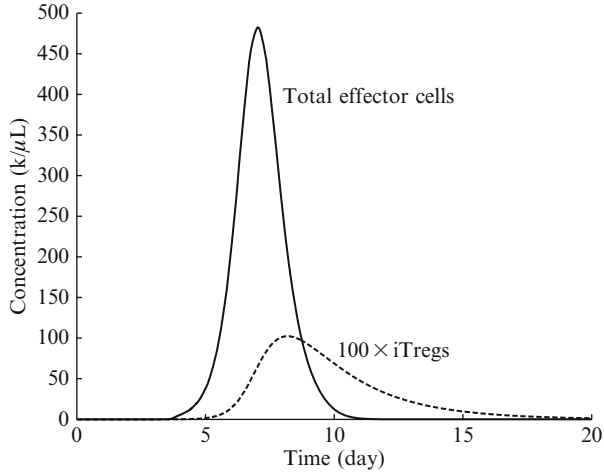


Figure 4.11 **Time evolution of effector and iTreg populations over time. The peak of the iTreg response roughly coincides with the peak of the T cell response, but the iTreg response decays slower.**

indicates that the iTreg concentration peaks around the same time as the T cell response, but lingers a while longer ensuring a full contraction of the T cell population. In this example, the naïve T cell population begins at 0.04 k/μL and peaks at 482 k/μL, corresponding to an expansion level of 13.6 divisions on average.

The numerical simulations show that both the T cell program and feedback regulation models exhibit similar insensitivity to the nature of antigen stimulation. However, the feedback model behaves differently from the program-based model with respect to variations in precursor frequency. Specifically, the feedback model significantly reduces variance in precursor concentration (by a cubed root power law), whereas the T cell program model directly translates variance in precursor concentration to variance in peak T cell levels (by a linear scaling law).

5. Concluding Remarks

By constructing mathematical models based on DDEs, we show how we can investigate two structurally distinct, regulatory networks for T cell dynamics. Using modeling, we readily determine the similarities and differences between the two models. In particular, we find that both networks have very low sensitivity to changes in the nature of antigen stimulation, but differ greatly in how they respond to variations in T cell precursor

frequency. Hence, our example demonstrates how mathematical and computational analysis can immediately provide a testable hypothesis to help validate or invalidate these two proposed regulatory networks.

Moving to a broader perspective, the entire immune response operates as a system of self-regulating networks, and many of these networks have the potential to be elucidated by mathematical modeling and computational simulation. Several modeling frameworks already exist and up to now, ODE models have been the most widely used due to their versatility across a wide range of problems and their ability to handle complex systems efficiently. DDEs and PDEs, which are both infinite-dimensional systems, also frequently appear in the repertoire of deterministic models. DDEs possess one advantage over ODEs in that they explicitly account for the delayed feedback without adding substantial computational complexity. PDE models provide an even more complex framework and can incorporate a wide range of spatial and temporal phenomena such as molecular diffusion and cell motion and maturation.

Among probabilistic models, stochastic ABMs are the most widely used, since they are typically easy to formulate, directly model individual diversity within populations, and recreate phenomena resulting from random events. An unavoidable disadvantage of ABMs is, however, that they are computationally demanding, especially in comparison to deterministic, differential equation models that can often be used to approximate the same phenomena with good accuracy. Thus, a promising compromise between the deterministic, differential equation, and stochastic agent-based paradigms comes from SDEs, a type of differential equation system that incorporates stochastic behavior. Nonetheless, this domain of mathematical modeling remains largely unexplored, at least for immunological networks, and offers a strong possibility for future research.

The immune regulatory network is intricate, made up of myriad intracellular and intercellular interactions that evade complete understanding and provide fertile ground for the unraveling of these interwoven mysteries with the help of insight gained from mathematical and computational modeling.

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REFERENCES

Antia, R., Bergstrom, C. T., Pilyugin, S. S., Kaech, S. M., and Ahmed, R. (2003). Models of CD8+ responses: 1. What is the antigen-independent proliferation program. *J. Theor. Biol.* **221**(4), 585–598.

Arstila, T. P., Casrouge, A., Baron, V., Even, J., Kanellopoulos, J., and Kourilsky, P. (1999). A direct estimate of the human alphabeta T cell receptor diversity. *Science* **286**(5441), 958–961.

Bains, I., Antia, R., Callard, R., and Yates, A. J. (2009). Quantifying the development of the peripheral naive CD4+ T-cell pool in humans. *Blood* **113**(22), 5480–5487.

Bheekha Escura, R., Wasserbauer, E., Hammerschmid, F., Pearce, A., Kidd, P., and Mudde, G. C. (1995). Regulation and targeting of T-cell immune responses by IgE and IgG antibodies. *Immunology* **86**(3), 343–350.

Böhm, C. M., Hanski, M. L., Stefanović, S., Rammensee, H. G., Stein, H., Taylor-Papadimitriou, J., Riecken, E. O., and Hanski, C. (1998). Identification of HLA-A2-restricted epitopes of the tumor-associated antigen MUC2 recognized by human cytotoxic T cells. *Int. J. Cancer* **75**(5), 688–693.

Burroughs, N. J., de Oliveira, B. M. P. M., and Pinto, A. A. (2006). Regulatory T cell adjustment of quorum growth thresholds and the control of local immune responses. *J. Theor. Biol.* **241,** 134–141.

Carneiro, J., Paixão, T., Milutinovic, D., Sousa, J., Leon, K., Gardner, R., and Faro, J. (2005). Immunological self-tolerance: Lessons from mathematical modeling. *J. Comput. Appl. Math.* **184,** 77–100.

Casadevall, A., and Pirofski, L. A. (2003). The damage-response framework of microbial pathogenesis. *Nat. Rev. Microbiol.* **1,** 17–24.

Casadevall, A., and Pirofski, L. A. (2006). A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. *Adv. Immunol.* **91,** 1–44.

Casal, A., Sumen, C., Reddy, T. E., Alber, M. S., and Lee, P. P. (2005). Agent-based modeling of the context dependency in T cell recognition. *J. Theor. Biol.* **236**(4), 376–391.

Catron, D. M., Itano, A. A., Pape, K. A., Mueller, D. L., and Jenkins, M. K. (2004). Visualizing the first 50 hr of the primary immune response to a soluble antigen. *Immunity* **21**(3), 341–347.

Celada, F., and Seiden, P. E. (1992). A computer model of cellular interactions in the immune system. *Immunol. Today* **13**(2), 56–62.

Colijn, C., and Mackey, M. C. (2005). A mathematical model of hematopoiesis—II. Cyclical neutropenia. *J. Theor. Biol.* **237**(2), 133–146.

De Boer, R. J., and Perelson, A. S. (1990). Size and connectivity as emergent properties of a developing immune network. *J. Theor. Biol.* **149**(3), 381–424.

De Boer, R. J., Kevrekidis, I. G., and Perelson, A. S. (1990). A simple idiotypic network with complex dynamics. *Chem. Eng. Sci.* **45,** 2375–2382.

de Pillis, L. G., Radunskaya, A. E., and Wiseman, C. L. (2005). A validated mathematical model of cell-mediated immune response to tumor growth. *Cancer Res.* **65**(17), 7950–7958.

Doumic-Jauffret, M., Kim, P. S., and Perthame, B. (2009). *Stability analysis of a simplified yet complete model for chronic myelegenous leukemia.* Submitted for publication.

Figge, M. T. (2009). Optimization of immunoglobulin substitution therapy by a stochastic immune response model. *PLoS ONE* **4**(5), e5685.

Figge, M. T., Garin, A., Gunzer, M., Kosco-Vilbois, M., Toellner, K. M., and Meyer-Hermann, M. (2008). Deriving a germinal center lymphocyte migration model from two-photon data. *J. Exp. Med.* **205**(13), 3019–3029.

Folcik, V. A., An, G. C., and Orosz, C. G. (2007). The Basic Immune Simulator: An agent-based model to study the interactions between innate and adaptive immunity. *Theor. Biol. Med. Model.* **4,** 39.

Fong, L., Hou, Y., Rivas, A., Benike, C., Yuen, A., Fisher, G. A., Davis, M. M., and Engleman, E. G. (2001). Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. *Proc. Natl. Acad. Sci. USA* **98**(15), 8809–8814.

Fouchet, D., and Regoes, R. (2008). A population dynamics analysis of the interaction between adaptive regulatory T cells and antigen presenting cells. *PLoS ONE* **3**(5), e2306.

Galon, J., Costes, A., Sanchez-Cabo, F., Kirilovsky, A., Mlecnik, B., Lagorce-Pagès, C., Tosolini, M., Camus, M., Berger, A., Wind, P., Zinzindohoué, F., Bruneval, P., *et al.* (2006). Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**(5795), 1960–1964.

Hackett, C. J., Rotrosen, D., Auchincloss, H., and Fauci, A. S. (2007). Immunology research: Challenges and opportunities in a time of budgetary constraint. *Nat. Immunol.* **8**(2), 114–117.

Heyman, B. (2000). Regulation of antibody responses via antibodies, complement, and Fc receptors. *Annu. Rev. Immunol.* **18,** 709–737.

Heyman, B. (2003). Feedback regulation by IgG antibodies. *Immunol. Lett.* **88**(2), 157–161.

Kaech, S. M., and Ahmed, R. (2001). Memory CD8+ T cell differentiation: Initial antigen encounter triggers a developmental program in naïve cells. *Nat. Immunol.* **2**(5), 415–422.

Kawakami, Y., and Rosenberg, S. A. (1997). Human tumor antigens recognized by T-cells. *Immunol. Res.* **16**(4), 313–339.

Kim, P. S., Lee, P. P., and Levy, D. (2009). *Emergent group dynamics governed by regulatory cells produce a robust primary T cell response.* Accepted by *Bull. Math. Biol.*

Kim, P. S., Lee, P. P., and Levy, D. (2007). Modeling regulation mechanisms of the immune system. *J. Theor. Biol.* **246,** 33–69.

Kim, P. S., Lee, P. P., and Levy, D. (2008). Modeling imatinib-treated chronic myelogenous leukemia: Reducing the complexity of agent-based models. *Bull. Math. Biol.* **70**(3), 728–744.

Lee, H. Y., Topham, D. J., Park, S. Y., Hollenbaugh, J., Treanor, J., Mosmann, T. R., Jin, X., Ward, B. M., Miao, H., Holden-Wiltse, J., Perelson, A. S., Zand, M., *et al.* (2009). Simulation and prediction of the adaptive immune response to influenza A virus infection. *J. Virol.* **83**(14), 7151–7165.

León, K., Lage, A., and Carneiro, J. (2003). Tolerance and immunity in a mathematical model of T-cell mediated suppression. *J. Theor. Biol.* **225,** 107–126.

León, K., Faro, J., Lage, A., and Carneiro, J. (2004). Inverse correlation between the incidences of autoimmune disease and infection predicted by a model of T cell mediated tolerance. *J. Autoimmun.* **22,** 31–42.

León, K., Lage, A., and Carneiro, J. (2007a). How regulatory CD25+CD4+ T cells impinge on tumor immunobiology? On the existence of two alternative dynamical classes of tumors. *J. Theor. Biol.* **247,** 122–137.

León, K., Lage, A., and Carneiro, J. (2007b). How regulatory CD25+CD4+ T cells impinge on tumor immunobiology: The differential response of tumors to therapies. *J. Immunol.* **179**(9), 5659–5668.

Mason, D. (1998). A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol. Today* **19**(9), 395–404.

Mata, J., and Cohn, M. (2007). Cellular automata-based modeling program: Synthetic immune system. *Immunol. Rev.* **216,** 198–212.

Meffre, E., and Wardemann, H. (2008). B-cell tolerance checkpoints in health and autoimmunity. *Curr. Opin. Immunol.* **20**(6), 632–638.

Mercado, R., Vijh, S., Allen, S. E., Kerksiek, K., Pilip, I. M., and Pamer, E. G. (2000). Early programming of T cell populations responding to bacterial infection. *J. Immunol.* **165**(12), 6833–6839.

Merrill, S. J. (1981). A model of the role of natural killer cells in immune surveillance—I. *J. Math. Biol.* **12,** 363–373.

Molldrem, J. J., Lee, P. P., Wang, C., Champlin, R. E., and Davis, M. M. (1999). A PR1-human leukocyte antigen-A2 tetramer can be used to isolate low-frequency cytotoxic T lymphocytes from healthy donors that selectively lyse chronic myelogenous leukemia. *Cancer Res.* **59**(11), 2675–2681.

Moore, H., and Li, N. K. (2004). A mathematical model for chronic myelogenous leukemia (CML) and T cell interaction. *J. Theor. Biol.* **225**(4), 513–523.

Murakami, M., Sakamoto, A., Bender, J., Kappler, J., and Marrack, P. (1998). CD25+CD4+ T cells contribute to the control of memory CD8+ T cells. *Proc. Natl. Acad. Sci. USA* **99** (13), 8832–8837.

Nelson, B. H. (2008). The impact of T-cell immunity on ovarian cancer outcomes. *Immunol. Rev.* **222,** 101–116.

Oka, Y., Elisseeva, O. A., Tsuboi, A., Ogawa, H., Tamaki, H., Li, H., Oji, Y., Kim, E. H., Soma, T., Asada, M., Ueda, K., Maruya, E., *et al.* (2000). Human cytotoxic T-lymphocyte responses specific for peptides of the wild-type Wilms' tumor gene (WT1) product. *Immunogenetics* **51**(2), 99–107.

Onsum, M., and Rao, C. V. (2007). A mathematical model for neutrophil gradient sensing and polarization. *PLoS Comput. Biol.* **3**(3), e36.

Pardoll, D. M. (1999). Inducing autoimmune disease to treat cancer. *Proc. Natl. Acad. Sci. USA* **96**(10), 5340–5342.

Rajewsky, K. (1996). Clonal selection and learning in the antibody system. *Nature* **381**(6585), 751–758.

Rosenberg, S. A. (2001). Progress in human tumour immunology and immunotherapy. *Nature* **411**(6835), 380–384.

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. (1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor a-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**(3), 1151–1164.

Sakaguchi, S., Yamaguchi, T., Nomura, T., and Ono, M. (2008). Regulatory T cells and immune tolerance. *Cell* **133**(5), 775–787.

Scherer, A., Salathé, M., and Bonhoeffer, S. (2006). High epitope expression levels increase competition between T cells. *PLoS Comput. Biol.* **2**(8), e109.

Seiden, P. E., and Celada, F. (1992). A model for simulating cognate recognition and response in the immune system. *J. Theor. Biol.* **158**(3), 329–357.

Shahaf, G., Johnson, K., and Mehr, R. (2005). B cell development in aging mice: Lessons from mathematical modeling. *Int. Immunol.* **18,** 31–39.

Sotiropoulou, P. A., Perez, S. A., Voelter, V., Echner, H., Missitzis, I., Tsavaris, N. B., Papamichail, M., and Baxevanis, C. N. (2003). Natural CD8+ T-cell responses against MHC class I epitopes of the HER-2/neu oncoprotein in patients with epithelial tumors. *Cancer Immunol. Immunother.* **52**(12), 771–779.

van Stipdonk, M. J., Hardenberg, G., Bijker, M. S., Lemmens, E. E., Droin, N. M., Green, D. R., and Schoenberger, S. P. (2003). Dynamic programming of CD8+ T lymphocyte responses. *Nat. Immunol.* **4**(4), 361–365.

Varela, F. J., and Stewart, J. (1990). Dynamics of a class of immune networks: Global stability of idiotype interactions. *J. Theor. Biol.* **144,** 93–101.

Weisbuch, G., DeBoer, R. J., and Perelson, A. S. (1990). Localized memories in idiotypic networks. *J. Theor. Biol.* **146**(4), 483–499.

Wodarz, D., and Thomsen, A. R. (2005). Effect of the CTL proliferation program on virus dynamics. *Int. Immunol.* **17**(9), 1269–1276.