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THEORETICAL STUDIES OF SELF-TOLERANCE : REGULATORY T CELLS AND ANERGY

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https://doi.org/10.15017/21712

出版情報：九州大学, 2011, 博士（理学）, 課程博士
バージョン：
権利関係：

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THEORETICAL STUDIES OF SELF-TOLERANCE: REGULATORY T CELLS AND ANERGY

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Submitted to the Graduate School of Kyushu University
in partial fulfillment of the requirements
for the degree of
Doctor of Science in Biology
Systems Life Sciences, Kyushu University
December 5, 2011

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Preface

Adaptive immunity of vertebrates is performed by a large repertoire of T and B lymphocytes, characterized by a diversity of receptors in order to recognize a variety of pathogens. The diversity of receptors is generated by somatic recombination during lymphocyte development (reviewed in Parham, 2009). Random somatic recombination inevitably produce lymphocytes that recognize proteins and other substances constituting a body (self-antigens). If these self-reactive lymphocytes become active in a body, they may cause autoimmunity -- immune system attacks a body itself. To avoid this, self-reactive lymphocytes must be removed.

A mechanism choosing lymphocytes with preferable receptors exits, described as clonal selection (Burnet, 1959). In the case of T cell development in the thymus, at first those with a minimum affinity to peptide-MHC (pMHC) complex are first positively selected because the interaction with pMHC is essential for T cell function. After the positive selection, survived thymocytes then undergo "negative selection" in which lymphocytes that recognize pMHC with high affinity are killed by apoptosis (Kappler et al., 1987). Clonal selection, especially negative selection, probably accounts for the largest reduction of the risk of autoimmunity, but it is unlikely to be complete. Some of the self-antigens might not be presented in the thymus, and even if all of the self-antigens were presented, it is also unlikely that each thymocytes checks all self-epitopes in the thymus concerning whether it can recognize or not during a limited selection period. It is suspected that self-reactive T cells are commonly present in the peripheral T cell repertoire (Anderton and Wraith, 2002). Therefore to prevent autoimmunity that is very harmful to the host body, there must be additional mechanisms to maintain self-tolerance in the periphery.

In this thesis I focuses on two mechanisms important for self-tolerance: regulatory T cells (chapter 1, 2) and anergy (chapter 3) and analyze these processes mathematically. Regulatory T cell is a subclass of $CD4^+$ T lymphocytes which plays an important role in the prevention of autoimmunity (Sakaguchi et al. 2008). They have a function to suppress

immune responses, and therefore many researches attempt to reveal the function of regulatory T cells for the clinical issues: for example the treatment and prevention of immunological diseases, the induction of transplantation tolerance, and the suppression of graft rejection. T cell anergy is defined as a state of unresponsiveness in T cells associated with nonproliferation and a lack of cytokine production (Schwartz, 2003). T cells are functionally inactivated following an antigen encounter, but remains alive for extended period of time. There are some theoretical studies on how these mechanisms can establish the self-tolerance (e.g., Carneiro et al. 2005). However they focused on the population dynamics of T cells, and did not consider the cost and the benefit to the host quantitatively. On the other hand, a few theoretical researches focused on discussing the advantage of designs of immune systems, except for Emi Shudo and her colleagues, who analyzed the adaptive significance of immune systems by comparing possible alternatives (Shudo and Iwasa, 2001, 2003, 2004; Shudo et al., 2003).

I discuss the adaptive significance of two mechanisms for self-tolerance by defining "fitness" explicitly. In the evolution based on natural seletion, the lifetime reproductive success, or the fitness, is improved, and hence we can expect that in the end of evolution we should observe the organism to maximize the fitness under the constraints. Hence we can use the fitness as a quantity to measure the success of alternative behaviors. The fitness defined in this thesis include the benefit of eliminating pathogens by activating pathogen specific effector T cells, and the harm of activating self-reactive T cells. By defining the fitness explicitly, we can compare the case with a tolerance mechanism and the case without it. Thus the model addresses the question of why a particular mechanism is adopted by the organisms rather than alternatives. The condition in which these tolerance mechanisms are beneficial also help us understand the condition in the body or which properties are regarded as important by the immune system. In the following I summarize the contents of chapters.

Chapter 1: Advantage of having regulatory T cells requires localized suppression of immune reactions

The immune system of vertebrates may attack its own body and cause autoimmunity diseases. To prevent autoimmumity, regulatory T cells suppress the activity of the autoreactive effector T cells, but they also interrupt normal immune reactions against foreign antigens. We discuss the advantage of having some regulatory T cells by considering the host's ability of coping with foreign antigens and the harm of autoimmunity. Assumptions are: The immature T cells reactive to abundant self antigens are eliminated, those reactive to rare self antigen will become regulatory T cells, and those that fail to interact with the antigens to which they are reactive will become effector T cells. Some self-reactive immature T cells may fail to interact with their own target antigens during the limited training period, and will later become to effector T cells, causing autoimmunity. Analysis suggests that, having some regulatory T cells can never be advantageous to the host, if activated regulatory T cells suppress effector T cells at any location of the body (global suppression). In contrast producing some regulatory T cells can be beneficial, if the body is composed of many compartments and regulatory T cells suppress the immune reactions only within the same compartment (localized suppression). This requires regulatory T cells to stop circulating once they are activated by their own target self antigens.

Chapter 2: Optimal number of regulatory T cells

The adaptive immune systems of vertebrates may attack their own body and cause autoimmunity diseases. Regulatory T cells suppress the activity of the autoreactive effector T cells, but they also interrupt normal immune reactions against foreign antigens. I discuss the optimal number of regulatory T cells to produce. Assumptions are: Some self-reactive immature T cells may fail to interact with their own target antigens during the limited training period, and will later become effector T cells, causing autoimmunity. There are

regulatory T cells that can recognize self-antigens. When a regulatory T cell is stimulated by its target self-antigen on an antigen presenting cell (APC), it stays there and suppresses the activation of other naive T cells on the same APC. Analysis of the benefit and the harm of having regulatory T cells suggests that the optimal number of regulatory T cells depends on the amount of self-antigens, severity of the autoimmunity, and the abundance of the pathogenic foreign antigens, and the spatial distribution of self-antigens in the body. If there are multiple types of self-antigens, we discuss the optimal number of regulatory T cells when self-antigens are localized in different sections of a body and when they are in the same section (co-localization). We also discuss the difference between when regulatory T cells for different self-antigens can be regulated separately and when they are constrained to be equal in number.

Chapter 3: T cell anergy as a strategy to reduce the risk of autoimmunity

Some self-reactive immature T cells escape negative selection in the thymus and may cause autoimmune diseases later. In the periphery, T cells stimulated insufficiently by peptide-major histocompatibility complex become inactive and their production of cytokines changes, a phenomenon called "T-cell anergy". We explore the hypothesis that T-cell anergy functions to reduce the risk of autoimmunity. The underlying logic is as follows: Since those self-reactive T cells that receive strong stimuli from self-antigens are eliminated in the thymus, T cells that receive strong stimuli in the periphery are likely to be non-self-reactive. As a consequence, when a T cell receives a weak stimulus, the likelihood that the cell is self-reactive is higher than in the case that it receives a strong stimulus. Therefore, inactivation of the T cell may reduce the danger of autoimmunity. We consider the formalism in which each T cell engages in Bayesian decision-making to reduce the risk of autoimmune diseases while maintaining its ability to attack non-self-antigens effectively. The optimal T cell responses to a weak and a strong stimulus are obtained both when the

cells respond in a deterministic manner and when they respond in a probabilistic manner. The conclusion is that T-cell anergy is the optimal response when a T cell meets with antigen-presenting cells many times in its lifetime, and when the product of the autoimmunity risk and the number of self-reactive T cells has an intermediate value.

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Chapter 1

Advantage of having regulatory T cells requires localized suppression of immune reactions

The study of this chapter was published in
Saeki, K., and Iwasa, Y., *Journal of Theoretical Biology*, **260**, 392-401 in 2009.

Introduction

Self-reactive naive T cells are mostly eliminated by the negative selection in the thymus (Kappler et al., 1987), but this process is not perfect and some of them exist in our bodies (Sakaguchi and Sakaguchi, 1990; Seddon and Mason, 1999; Jordan et al., 2001; Moon et al. 2011). These self-reactive T cells are prevented from being activated by T cell ignorance (Ohashi et al., 1991), T cell anergy (Schwartz, 1997), or regulatory T cells (Sakaguchi et al. 2004). A subclass of T lymphocites, called regulatory T cells, suppresses pathological and physiological immune responses plays an important role in preventing the autoimmunity. For example, the depletion of those T cells produces autoimmune diseases and their reconstitution prevents the diseases (Sakaguchi, 1995). Regulatory T cells express the transcription factor forkhead box P3 (FOXP3) and most of them are $CD4^+$ T cells that express CD25 (the interleukin-2 (IL-2) receptor α-chain). They are developmentally classified into natural and induced, the former are produced by the normal thymus as a functionally mature and distinct population and the latter are induced from naive T cells by specific modes of antigenic stimulation, especially in a particular cytokine milieu (Roncarolo et al., 2006). In this and next chapter, the model considers the naturally occurring regulatory T cells. The point is that natural Tregs are produced in the thymus by recognizing self-epitopes, which means that they can recognize epitopes derived from self-antigens.

Activated regulatory T cells can suppress wide range of immune cells including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NKT cells, B cells and antigen-presenting cells (APCs) *in vitro* and *in vivo* (Sakaguchi et al., 2008). The mechanisms of suppression are grouped into some modes: suppression by inhibitory cytokines, suppression by cytolysis, suppression by metabolic disruption and suppression by modulation of dendritic-cell (DC) maturation or function (reviewed in Vignali et al. 2008, Shevach 2009). Whereas regulatory T cells control immune response and prevent autoimmunity, they suppress irrespective of antigen specificity of target immune cells (Thornton and Shevach, 2000) and the possibility interrupting immune reactions against pathogens or cancer cells has been discussed (Cools et

al., 2007). In deed, recent researches show that pathogen specific regulatory T cells may disturb the early T cell response (Shafiani et al. 2010) and the existence of pathogen specific regulatory T cells (Zhao et al. 2011).

Many theoretical studies have focused on the question of how this relatively "non-specific" regulatory mechanism permits a proper balance between tolerance to self-antigens and immunity to foreign antigens. Leon et al. (2000, 2003) and Carneiro et al. (2007) modeled the conjugates of regulatory T cells, conventional effector T cells, and APCs including the thymic generation and the peripheral dynamics of T cell clones. They found that natural tolerance is based on ubiquitous and constitutive self-antigens, which select and sustain clones of specific regulatory T cells, both positive and negative selections are required to establish a proper anti-self-regulatory T cells. Burroughs et al. (2006, 2008) modeled cytokine dependent growth of T cells and inhibition of IL-2 secretion by regulatory T cells. The state of effector T cell population becomes either controlled or activated, and non-specific inhibition is prone to the escape of initially controlled autoreactive effector T cell through cross reactivity to pathogens. These studies have been focused on the dynamics of T cell population rather than explicitly considering the cost and the benefit to the host quantitatively.

Here I discuss the advantage of having regulatory T cells by considering the ability of coping with foreign antigens and the harm of autoimmunity. For simplicity, some major assumptions are set, that is, 1) T cells are not cross-reactive and only recognize their cognate epitopes, 2) T cells are classified into "self-reactive" and "non-self-reactive". Additionally, in this section the mechanism of suppression depends on direct interactions between regulatory T cells and conventional T cells, which is achieved, for instance, by Granzyme B (Gondek et al. 2005) or CD95-CD95 ligand (Strauss et al. 2009)

Model

Benefit and harm of immune reactions

To discuss the benefit of having regulatory T cells, I use the fitness which describes the ability to survive and reproduce. In this study, fitness is consisted of three additive parts: 1) a basic part which is not affected by the existence of T cells, 2) positive effect by eliminating pathogens, and 3) negative effect by autoimmunity. I assume that both positive and negative effects are proportional to the abundance of effector T cells, and the fitness is expressed as follows

$$\Phi = \Lambda + \nu \begin{bmatrix} \text{abundance of} \\ \text{non - self - reactive} \\ \text{effector T cells} \end{bmatrix} - \mu \begin{bmatrix} \text{abundance of} \\ \text{self - reactive} \\ \text{effector T cells} \end{bmatrix} \qquad (1.1)$$

where Λ is the basic fitness without any T cells, ν is the benefit by a non-self-reactive effector T cells, and μ is the severity of autoimmunity by a self-reactive effector T cells. Effector T cells cause the benefit of immune reaction against foreign antigens represented by the second term, and the harm caused by autoimmune reactions represented by the third term.

Procedure of T cell development

In the first step of T cell development, positive selection in the thymus selects immature T cells that can recognize a peptide-MHC complex with a certain affinity. These surviving immature T cells are composed of: [1] cells reactive to common self-antigens, [2] cells reactive to rare self-antigens, and [3] cells not reactive to any of the self-antigens. Self-reactive T cells belonging [1] and [2] are dangerous but T cells in the class [3] is likely to be useful for fighting against foreign antigens. Note that there are T cells reactive to common epitopes between self-antigens and foreign antigens, which are classified into self-reactive T cells.

In the next step, the negative selection and the induction of regulatory T cells are

conducted. At first, these processes are assumed to occur step by step, that is, negative selection followed by differentiation (Fig. 1. 1). The other possibilities are considered later. Here I assume that all the cells in class [1] are eliminated because almost all the immature T cells can meet with abundant self-epitopes. However, those cells belonging to class [2] survive due to the rareness of their target self-epitopes. Of course, cells belonging to class [3] survive because they never recognize any kind of self-epitopes in the thymus. Immature T cells are determined to be regulatory T cells or to be effector T cells based on whether or not they have interacted with their own target antigens during the differentiation step (Fig. 1. 1).

Suppose the number of times a particular self-reactive immature T cell to meet with its corresponding epitope during the training period follows a Poisson distribution with mean a, where a is the effectiveness of training procedure for rare self-antigens. Then with probability e^{-a} the cell does not encounter with the epitope but with probability $1 - e^{-a}$ the cell recognize. Let N_S be the number of immature T cells reactive to rare self-epitopes. By the differentiation step, $N_S(1 - e^{-a})$ immature T cells become regulatory T cells, and $N_S e^{-a}$ immature T cells become self-reactive effector T cells, which will cause autoimmunity. In contrast N_F immature T cells are not stimulated during the differentiation period, and become effector T cells, which can be reactive to pathogenic foreign antigens.

Suppression by regulatory T cells

When a regulatory T cell recognizes a self-epitope to which it is reactive in the periphery, it is activated and starts suppressing the immune reactions. As mentioned above, here I consider the case in which the suppression by activated regulatory T cells occurs via cell-to-cell interaction with effector T cells. If T cells encounter each other at random, the number of times for a particular effector T cell to meet with regulatory T cells follows a Poisson distribution with mean proportional to the number of activated regulatory T cells, where the proportionality coefficient is λ named encounter efficiency. Hence the probability of being not suppressed is $e^{-\lambda N_S\left(1-e^{-a}\right)}$.

Fitness

After modeling the abundance of each type of T cells and the probability of suppression, the fitness given by Eq. (1. 1) becomes,

$$\begin{aligned}\Phi &= \Lambda + \nu N_F e^{-\lambda N_S\left(1-e^{-a}\right)} - \mu N_S e^{-a} e^{-\lambda N_S\left(1-e^{-a}\right)} \\ &= \Lambda + \nu N_F \left(1 - \frac{\mu N_S}{\nu N_F} e^{-a}\right) e^{-\lambda N_S\left(1-e^{-a}\right)}\end{aligned} \tag{1. 2}$$

Ratio μ/ν indicates the relative severity of autoimmunity to the benefit of attacking foreign antigens, and $N_S e^{-a}/N_F$ is the relative abundance of self-reactive effector T cells. If there are no regulatory T cells and all the immature T cells that interacted with self-epitopes are eliminated, the fitness would be

$$\Phi^0 = \Lambda + \nu N_f \left(1 - \frac{\mu N_s}{\nu N_f} e^{-a}\right) \tag{1. 3}$$

When $\nu N_F > \mu N_S e^{-a}$, $\Phi < \Phi^0$ holds, implying that producing regulatory T cells is not beneficial to the host. When instead $\nu N_F \le \mu N_S e^{-a}$, $\Phi \le \Lambda$ hold, implying that T cells cause more harm than benefit. Taken together, there is no possibility for producing regulatory T cells to be adaptive.

Localized suppression by regulatory T cells

Since regulatory T cells activated suppress effector T cells irrespective of their antigen specificity, the fractions of suppressed cells are same between self-reactive effector T cells and foreign-antigen-reactive effector T cells. This is the reason why producing regulatory T cells is not beneficial. I here include the compartimentalization of a body, which makes regulatory T cells suppress self-reactive effector T cells more than useful effector T cells reactive to foreign antigens. Assumptions are: a body is composed of a large number of local sites, immune cells are circulating among these, and regulatory T cells stay within the local

site when they recognize their cognate self-epitopes (localized suppression). The point is that an immune response against foreign antigens occurs in every site, but an autoimmune response and the suppression of effector T cells occurs in sites which include rare self-antigens. Let each site include $rN_S(1 - e^{-a})$ regulatory T cells ($0 < r << 1$), and let q be the fraction of sites with rare self-antigens. The fitness consists of the basal fitness and the average of the benefit and cost calculated over different compartments, where the advantage and disadvantage over different site are combined additively,

$$\begin{aligned}\Phi &= \Lambda + (1-q)\nu N_F + q\left(\nu N_F - \mu N_S e^{-a}\right) e^{-\lambda r N_S\left(1-e^{-a}\right)} \\ &= \Lambda + (1-q)\nu N_F \left(1 + \frac{q}{1-q}\left(1 - \frac{\mu N_S}{\nu N_F} e^{-a}\right) e^{-\lambda r N_S\left(1-e^{-a}\right)}\right)\end{aligned} \tag{1.4}$$

where the second term is the fitness in sites without rare self-antigens, and the third term is that with rare self-antigens. When no regulatory T cells are produced, the fitness would be

$$\Phi^0 = \Lambda + (1-q)\nu N_F \left(1 + \frac{q}{1-q}\left(1 - \frac{\mu N_S}{\nu N_F} e^{-a}\right)\right) \tag{1.5}$$

As in the above $\nu N_F > \mu N_S e^{-a}$ is necessary for $\Phi > \Phi_0$, which is very important condition also in the latter sections. For $\Phi > \Lambda$,

$$1 + \frac{q}{1-q}\left(1 - \frac{\mu r N_S}{\nu N_F} e^{-a}\right) e^{-\lambda r N_S\left(1-e^{-a}\right)} > 0$$

should hold, which is rewritten as

$$q < \left(1 - \left(1 - \frac{\mu N_S}{\nu N_F} e^{-a}\right) e^{-\lambda r N_S\left(1-e^{-a}\right)}\right)^{-1} \tag{1.6}$$

For producing regulatory T cells to be beneficial, the fraction of sites that contain self-antigens q should be enough small, which matches the definition of "rare" self-antigens. In addition the effect of the effectiveness of training procedure a seems to be not monotonic because increasing a results the reduction of self-reactive T cells but it violates $\nu N_F > \mu N_S e^{-a}$. Fig. 1. 2 indicates the region in which regulatory T cells are beneficial on the a-q plane (shown by gray). Although I don't know the realistic values of parameters, rare self-antigens

is expected to have low a and q. Looking along the horizontal axis, both too small and too large a do not satisfy the condition for producing regulatory T cells to be beneficial.

When the cell fate is determined before the training period

The development of T cells was assumed that immature T cells differentiate to regulatory T cells or effector T cells after they experience training, but an alternative situation is possible that is T cell fate is determined before the training period. I call the first model "experience-dependent differentiation", and this new model "pre-determined differentiation". In this model, at first some fraction b of immature T cells that are not in class [1] are determined to be regulatory T cells and the others are determined to be effector T cells. If b is set to 0, no regulatory T cells are produced, and if b = 1, all immature T cells become regulatory T cells, which makes no immune reactions. After the determination of cell fates each kinds of cells has a different selection process (Fig. 1. 3). Effector T cell candidates are killed if they recognize their own target epitopes during the training period but they survive if they do not. In contrast regulatory T cell candidates survive if they recognize self-epitopes otherwise they are killed.

According to the processes in Fig. 1. 3, the numbers of regulatory, self-reactive, and non-self-reactive T cells are expressed respectively, $N_S b\left(1-e^{-a}\right)$, $N_S (1-b)e^{-a}$, and $N_F(1-b)$. In the case of the global suppression, the fitness is expressed as

$$\Phi = \Lambda + \nu N_F\left(1-\frac{\mu N_S}{\nu N_F}e^{-a}\right)(1-b)e^{-\lambda N_S b\left(1-e^{-a}\right)} \qquad (1.7)$$

This is a monotonic function of b in the interval $0 < b < 1$. If $\nu N_F > \mu N_S e^{-a}$, the optimal b that maximizes Eq.(1. 7) is zero, implying no regulatory T cells are needed. Instead if $\nu N_F \le \mu N_S e^{-a}$, Φ is always smaller than the basal fitness, Λ. Taken together, there is no possibility that some regulatory T cells are to be produced at the optimum as shown in the

experience-dependent differentiation model.

Since the difference from the experience-dependent differentiation model is the number of each type of t cells, the fitness with localized suppression is modification of Eq.(1. 4),

$$\Phi_{pre} = \Lambda + (1-q)\nu N_F(1-b)\left(1 + \frac{q}{1-q}\left(1 - \frac{\mu N_S}{\nu N_F}e^{-a}\right)e^{-\lambda r b N_S(1-e^{-a})}\right) \quad (1.8)$$

where q and r is same as before. For the analysis, two constants are introduced,

$$\alpha = \lambda r N_S(1-e^{-a}) \quad \text{and} \quad \beta = \frac{-q}{1-q}\left(1 - \frac{\mu N_S}{\nu N_F}e^{-a}\right)$$

where $\beta > 0$ because $\nu N_F < \mu N_S e^{-a}$ must hold as mentioned before. The optimal fraction of regulatory T cell candidates b^* which maximizes Eq.(1. 8) is obtained by examining the derivative,

$$\frac{d\Phi_{pre}}{db} = (1-q)\nu N_F\left[(1+(1-b)\alpha)\beta e^{-b\alpha} - 1\right] \quad (1.9)$$

From the sign of the right hand side of Eq.(1. 9), there are three possibilities about b^*: 1) b^* = 0 when $\beta \le (1+\alpha)^{-1}$, 2) $0 < b^* < 1$ when $(1+\alpha)^{-1} < \beta < e^{\alpha}$, and 3) $b^* = 1$ when $\beta \ge e^{\alpha}$. Note that producing regulatory T cells is beneficial only in the second case. The b^* is numerically calculated by increasing b from 0 to 1 in 0.001 increments on α-β plane (Fig. 1. 4). In the region for $0 < b^* < 1$, b^* is close to 0 near $\beta = (1+\alpha)^{-1}$, and it is close to 1 near $\beta = e^{\alpha}$.

Comparison with experience-dependent model

Using α and β enables to simplify the fitness of experience-dependent model Eq.(1. 4) as

$$\Phi_{\exp} = \Lambda + (1-q)\nu N_F(1-\beta e^{-\alpha}) \quad (1.10)$$

and the fitness of pre-determined differentiation model Eq.(1. 8) is

$$\Phi_{pre} = \Lambda + (1-q)\nu N_F(1-b^*)(1-\beta e^{-\alpha b^*}) \quad (1.11)$$

where b^* is the optimal differentiation rate of regulatory T cell that maximizes Φ_{pre}. The

difference of these two is

$$\Phi_{\exp} - \Phi_{pre} = (1-q)\nu N_F\left[b^* + \beta\left((1-b^*)e^{-\alpha b^*} - e^{-\alpha}\right)\right] \qquad (1.12)$$

If $0 < b^* < 1$ and $\beta > 0$, the following relationship is obtained from $(1+\alpha)^{-1} < \beta < e^{\alpha}$, which is the condition for $0 < b^* < 1$,

$$\begin{aligned}\Phi_{\exp} - \Phi_{pre} &> (1-q)\nu N_F\left[b^* + \beta\left((1-b^*)e^{-\alpha} - e^{-\alpha}\right)\right] \\ &= (1-q)\nu N_F b^*\left(1-\beta e^{-\alpha}\right) > 0\end{aligned} \qquad (1.13)$$

This implies that the experience-dependent differentiation is more advantageous than the pre-determined case as long as regulatory T cells are required. Hence, I conclude that the experience-dependant differentiation model is always superior to the pre-determined differentiation model with the same parameters.

Inducing selection and differentiation at the same time

I have assumed that immature T cells reactive to common self-antigens are eliminated, but it is difficult to tell each immature T cell whether it is reactive to common self-antigen, or to rare self-antigen or, to no self-antigen unless it experiences the education in the thymus. Next I hypothesize that being reactive to common self-antigens is distinguished from being reactive to rare ones based on the number of recognition of self-epitopes in the thymus. Thus a new process of education in the thymus is considered: an immature T cell which recognize self-epitopes during the training period will differentiate to a regulatory T cell, but in each interaction, the cell is killed with probability, *c*, (the scheme is shown in Fig. 1. 5). If an immature T cell interacts with its cognate self-epitopess many times, the cell is more likely to be killed. This is an expansion of experience-dependent differentiation model, and indicates that the negative selection and the induction of regulatory T cells are conducted at the same time. The fraction of T cells which will differentiate to the regulatory T cell is

$$\sum_{k=1}^{\infty} \frac{a^k}{k!} e^{-a} \left(1-c\right)^k = e^{-ac} - e^{-a} \tag{1.14}$$

When $c = 1$, cells that interacted with self-epitopes will surely be killed, and no regulatory T cells are produced. When $c = 0$, the situation is same as the experience-dependent differentiation in the previous section.

Suppose that there are two kinds of self antigens indexed by i ($i = 1$ or 2), which are different in the training efficiency (a_i) and the abundance in the periphery (q_i) and the severity of autoimmunity (μ_i), but the numbers of immature T cells reactive to them are the same between different types of self antigens (set $N_1 = N_2 = N/2$). The number of matured regulatory T cells are calculated by Eq.(1.14). With global suppression, the fitness is

$$\Phi = \Lambda + \nu N_F \left(1 - \frac{N_S}{2\nu N_F}\left(\mu_1 e^{-a_1} + \mu_2 e^{-a_2}\right)\right) \exp\left(-\lambda \frac{N_S}{2}\left(e^{-a_1 c} - e^{-a_1} + e^{-a_2 c} - e^{-a_2}\right)\right) \tag{1.15}$$

When the second factor in the right hand side is negative, the optimal death rate per interaction in the thymus, c^*, is zero but $\Phi > \Lambda$. When it is positive, $c^* = 1$ and immature T cells are always killed by recognizing self-antigens in the thymus. Thus, the global suppression cannot make producing regulatory T cells beneficial.

In the case of the localized suppuression, there are four types of sites: sites without self-antigens, sites only with common self-antigens, sites only with rare antigens, and sites with both type of self-antigens. From Eq.(1. 4), the fitness with localized suppression becomes,

$$\Phi = \Lambda + \left(1-q_1\right)\left(1-q_2\right)\nu N_F \phi[c] \tag{1.16}$$

where

$$\begin{aligned} \phi[c] = 1 &+ \sum_{i=1}^{2} Q_i S_i \exp\left(-\gamma\left(e^{-a_i c} - e^{-a_i}\right)\right) \\ &+ Q_1 Q_2 \left(S_1 + S_2 - 1\right) \exp\left(-\gamma\left(e^{-a_1 c} - e^{-a_1} + e^{-a_2 c} - e^{-a_2}\right)\right) \end{aligned} \tag{1.17}$$

and $Q_i = q_i/\left(1-q_i\right)$, $S_i = 1 - \left(\mu_i N_S / 2\nu N_F\right) e^{-a_i}$, $\gamma = r\lambda N_S/2$. Large Q_i implies that the i-th self-antigen is common, and $S_i < 0$ implies that attacking the i-th self-antigen is severer than

the harm of foreign antigens. The analytical calculation for the optimal death rate per interaction, c^*, is messy. Here I assume that self-antigen 1 ($i = 1$) is common and self-antigen 2 ($i = 2$) is rare, which implies that $q_1 > q_2$, and $a_1 > a_2$, and c^* is found numerically by increasing c from 0 to 1 in 0.001 increments. Fig.1. 6 illustrates c^* for different values of the severities of self-antigens, μ_1 and μ_2. There is a region in which $c^* = 0$ but $\Phi > \Lambda$. This suggests that producing regulatory T cells and no deletion is the optimal in some conditions. For the intermediate c^*, $S_I > 0$ should hold, which implies that the harm of attacking common self-antigen should be lower than the advantage of eliminating foreign antigens.

Discussion

In this chapter, I discussed the advantage of having regulatory T cells by considering the benefit of T cells that can respond to foreign antigens and the harm of autoimmunity. The fitness advantage of having T cells is determined by the abundance of effector T cells reactive to foreign or to self-antigens or to both escaping the suppression by regulatory T cells. The major result is that the localized suppression is essential for having regulatory T cells to be advantageous. To encounter and neutralize foreign antigens, activated effector T cells are likely to circulate among peripheral tissues. If regulatory T cells behave the same, the suppressive function of regulatory T cells does not affect the fitness-maximization. Therefore activated regulatory T cells should stay the site where they found their cognate self-antigens and suppress immune reactions only in their neighborhood. Mechanisms to suppress self-reactive effector T cells preferentially are reported, for example, the suppression on antigen presenting cells or the use of short-range suppressive humoral factor, interleukin-10 and transform growth factor β (reviewed in Sakaguchi et al. 2009). The need for localized suppression obtained by the simplest model also holds in the other differentiation scenarios.

However, the localized suppression does not always allow producing regulatory T cells

to be beneficial. To make regulatory T cells beneficial, self-antigens should be very dangerous and have low frequency in the periphery. This conclusion predicts that the target antigens of regulatory T cells are tissue specific antigens, or antigens expressed during the limited period in the life. In addition if the training for immature T cells is perfect and e^{-a} is 0, having regulatory T cells is also not beneficial. In this case, there are no self-reactive T cells, and regulatory T cells don't have to suppress immune reactions. Consistent with the conclusions of other theoretical studies, regulatory T cells seems to be required because the training of immature T cells are imperfect (Mason, 1998; Muller and Bonhoeffer, 2003; Stibor et al., 2006).

Defining fitness enables to compare the different scenarios of differentiation of regulatory T cells. The experience-dependent differentiation model suggests that immature T cells become effector T cells if they have not interacted with self-antigens in the thymus, and become regulatory T cells if they have interacted. In contrast, pre-determined model assumes that immature T cells are determined to be regulatory T cells or effector T cells at first, and the selection occurs afterward. The comparison of the fitness for these two models conclude that immature T cells should differentiate based on their experience during the training period. Hence, I expect that the development of regulatory T cells in the thymus depends on interactions with self-antigens. Several experiments support this idea that interactions with self-epitopes in the thymus enhances the differentiation to regulatory T cells (e.g., Hsieh et al., 2006; Jordan et al., 2001). However there might be additional constraints that force the host to take the determination of cell fate before the training period. I would like to note that there is a report that claims the stochastic determination of T cell fate (van Santen et al., 2004).

In addition, I also propose a mechanism to distinguish T cells reactive to common and rare self-antigens. Since there are very abundant self-antigens, too many regulatory T cells would be produced and would totally suppress the beneficial effector T cells acting on foreign antigens. To avoid this, at first I assumed that T cells reactive to abundant

self-antigens are perfectly eliminated. By introducing the probability of cell death per interaction in the training period, common self-antigen specific T cells are removed from the T cell repertoire but rare self-antigen specific T cells would become regulatory T cells.

For simplicity, I didn't include two aspects about T cell population: the cross-reactivity of T cell receptor, and the antigen-dependent clonal expansion in the periphery. The former changes the number of effector T cells reactive to self-antigens because T cells that can recognize both self and foreign antigens as self-reactive T cells. Some studies have examined a relationship between the cross-reactivity and the diversity of lymphocytes or a trade-off between responsivenesss to foreign antigens and unresponsivenesss to self-antigens (Perelson and Oster, 1979; De Boer and Pelelson, 1993; Nenazee, 1996; Borghans et al., 1999; Faro et al. 2004). Particularly, Mason (1998) pointed out that there is the optimal value of crossreactivity and that regulatory T cells are important to defend self-antigens from T cells reactive to both foreign and self antigens. However since the main purpose is to present the framework of cost-benefit thinking in understanding regulatory T functions, I believe that presenting the simplest possible model would be better than showing more complicated cases. In spite of many limitations, the concept of the advantage to the host of having regulatory T cells would give us an insight into the process of T cell maturation, and it can be useful in understanding the biology of regulatory T cells.

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Figure Legends

Figure 1. 1 The scheme of T cell maturing process in the first model. After the positive selection, self-reactive immature T cells are determined to be regulatory T cells or to be effector T cells based on whether or not they have interacted with self-antigens during the training period in the thymus. Those immature T cells have a chance to interact with a self-antigen at least once, which is expected as e^{-a}. In contrast non-self-reactive T cells never recognize any self-antigens and become effector T cells (not shown).

Figure 1. 2 The dependence of the condition for producing regulatory T cells to be beneficial on the frequency of self-antigens, q, and the effectiveness of training procedure for rare self-antigens, a. Gray shows the region in which both $\nu N_F > \mu N_S e^{-a}$ and inequality (1. 6) are satisfied, where $\mu N_S / \nu N_F = 100$ and $rN_S = 10$. In this case $\nu N_F > \mu N_S e^{-a}$ hold with $a <$ 4.60517.

Figure 1. 3 The scheme of T cell maturing process in the pre-determined model. Some self-reactive immature T cells are determined to be regulatory T cells with probability b, and others are determined to be effectors. After that, each T cell candidates is selected depending on the interaction with self-antigens. For the non-self-reactive T cells, fraction 1-b of them becomes effector T cells, and other fraction will die.

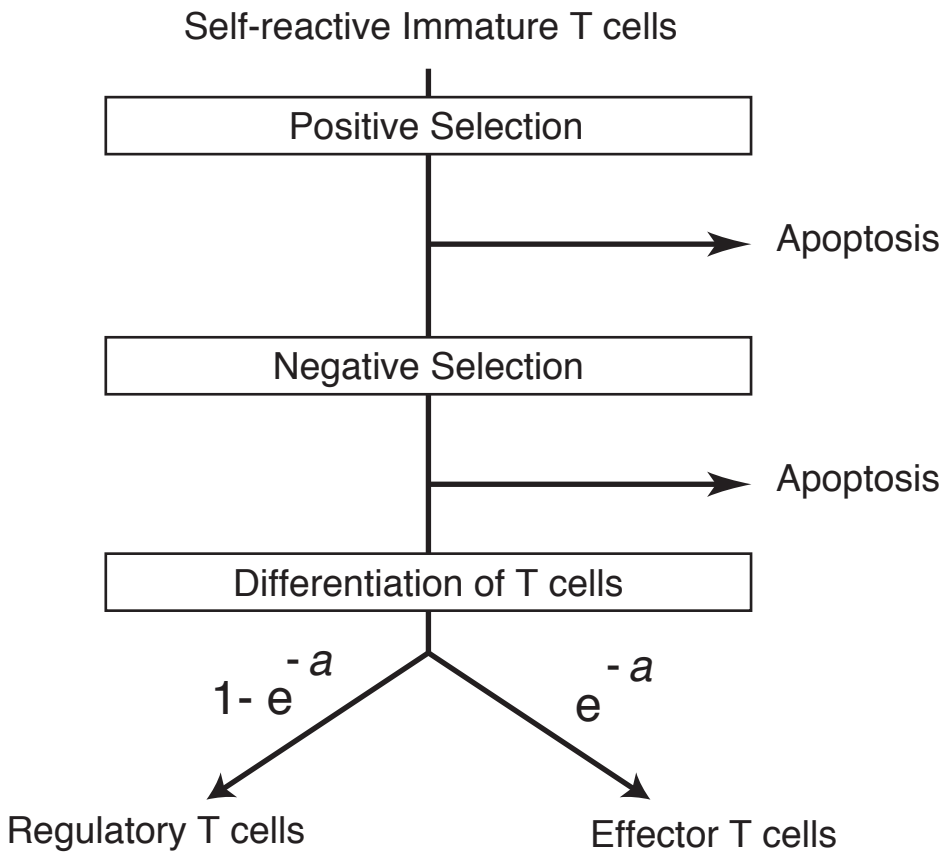
Figure 1. 4 The optimal differentiation rate of regulatory T cells, b^* that maximizes Eq.(1. 8) are numerically calculated and shown on α-β plane. Contours are plotted by every 0.25 degrees (broken lines). The boundary between $b^*=1$ and $0< b^*<1$ is equal to $\beta = e^{\alpha}$ and the boundary between $0< b^*<1$ and $b^*=0$ is equal to $\beta = (1+\alpha)^{-1}$ (shown as thick lines).

Figure 1. 5 The scheme of T cell maturing process in the experienced-dependent model with death rate per interaction, c. The differentiation to regulatory T cells and the induction

of apoptosis may occur at the same time. The probability that a self-reactive immature T cell does not recognize any self-antigens in the thymus is still e^{-a}. Among those interacted with self-antigens, some are killed, the other become regulatory T cells.

Figure 1.6 The optimal death rate per interaction, c^* that maximizes Eq.(1. 16) with two types of self-antigens. I assumed that common self-antigen has $a_1 = 4$ and $q_1 = 0.9$, and that rare self-antigen has $a_2 = 3$ and $q_2 = 0.1$. Then c^* is numerically calculated for each pair of μ_1 and μ_2, where $N_S/\nu N_F = 2$. In this case, $S_1 > 0$ when $\mu_1 < 5.45982$, and $S_2 > 0$ when $\mu_2 < 2.00855$. There are four regions: 1) $c^* = 0$ but $\Phi > \Lambda$, 2) $c^* = 0$, 3) c^* is intermediate, and 4) $c^* = 1$. The boundaries between two regions are shown by thick lines. Contours of c^* in third region are plotted by every 0.25 degrees (broken lines).

Figure 1. 1


Self-reactive Immature T cells
Positive Selection
Apoptosis
Negative Selection
Apoptosis
Differentiation of T cells
1- e - a
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Regulatory T cells
Effector T cells

Figure 1. 2

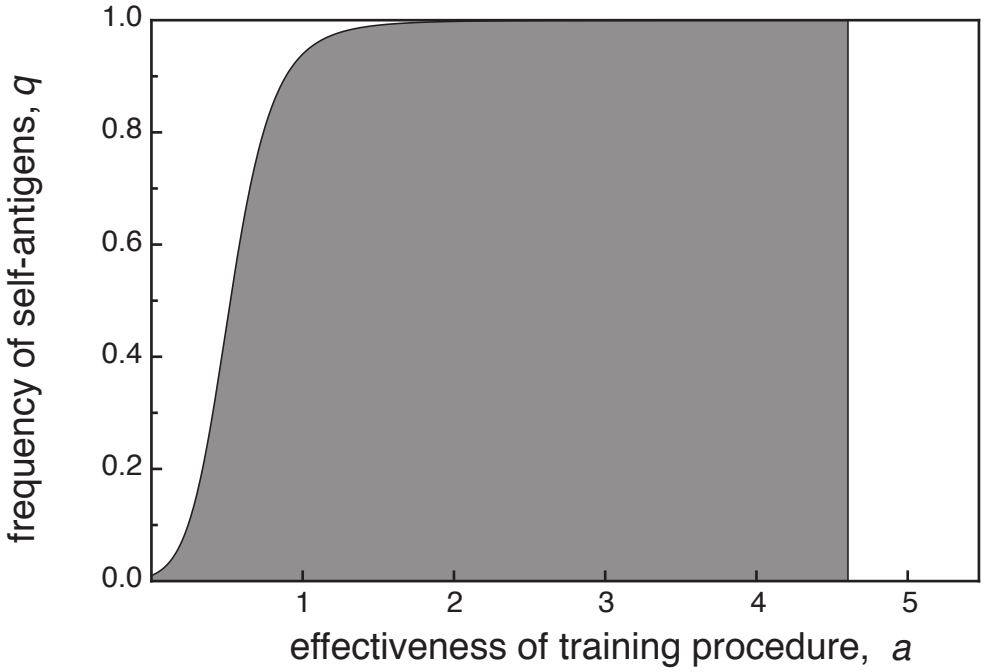

1.0
0.8
0.6
0.4
0.2
0.0
frequency of self-antigens, q
1
2
3
4
5
effectiveness of training procedure, a

Figure 1. 3

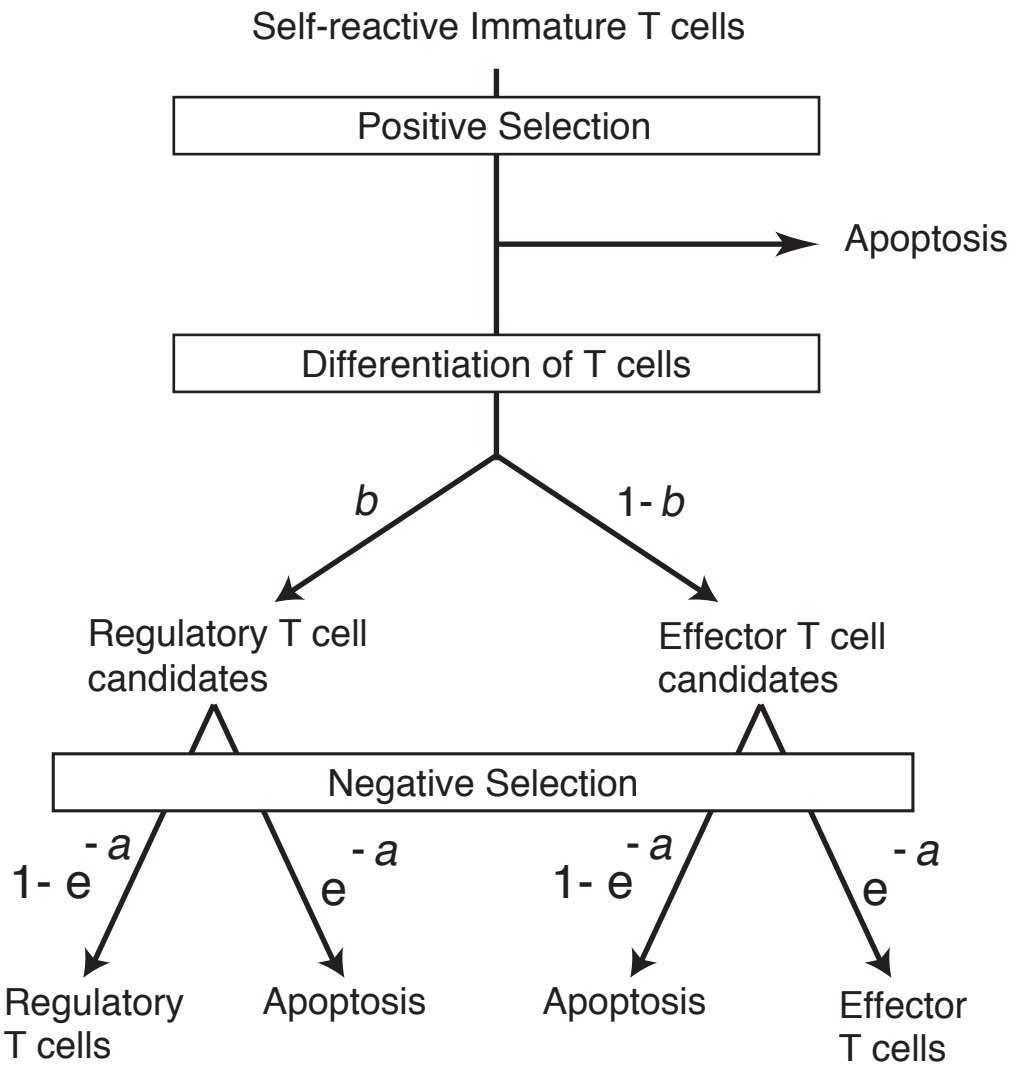

Self-reactive Immature T cells
Positive Selection
Apoptosis
Differentiation of T cells
b
1- b
Regulatory T cell candidates
Effector T cell candidates
Negative Selection
1- e - a
e - a
1- e - a
e - a
Regulatory T cells
Apoptosis
Apoptosis
Effector T cells

Figure 1. 4

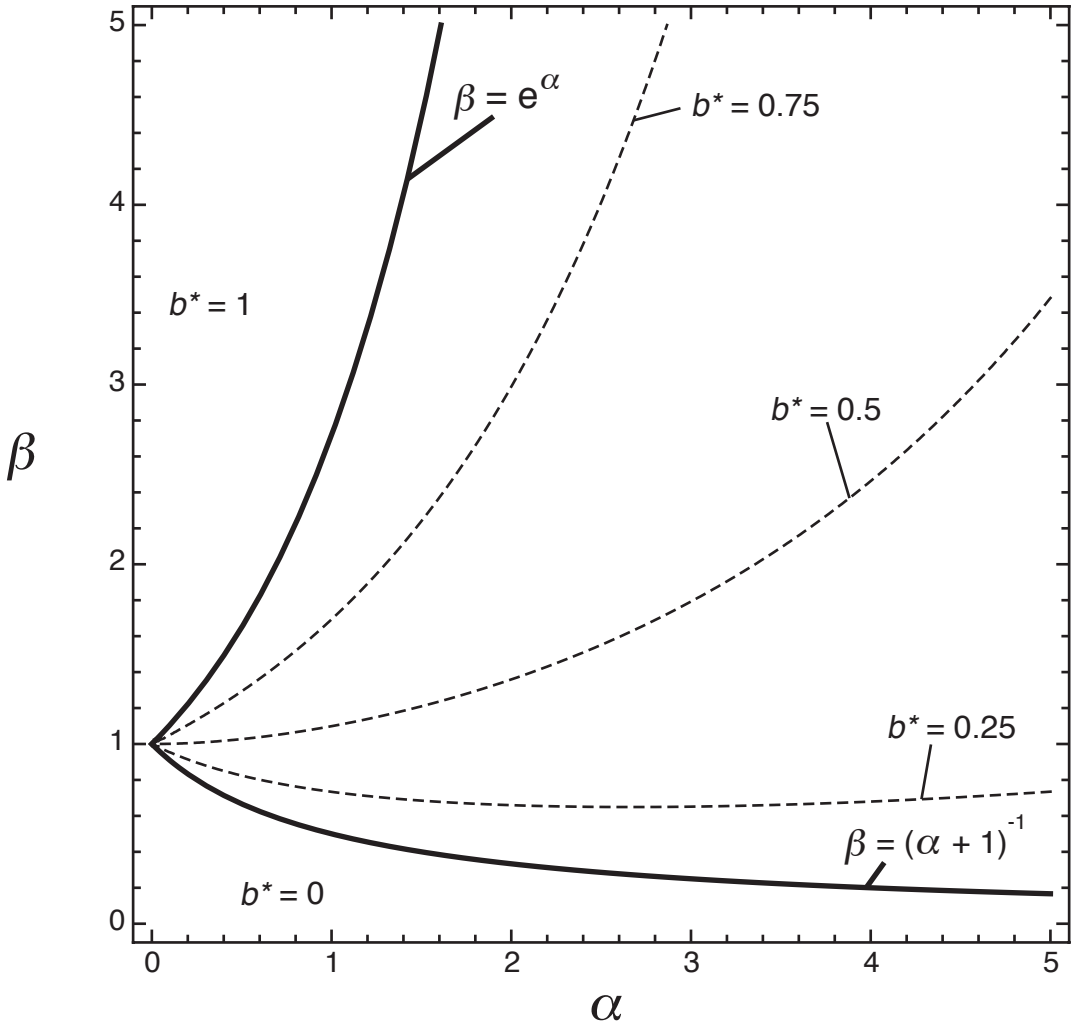
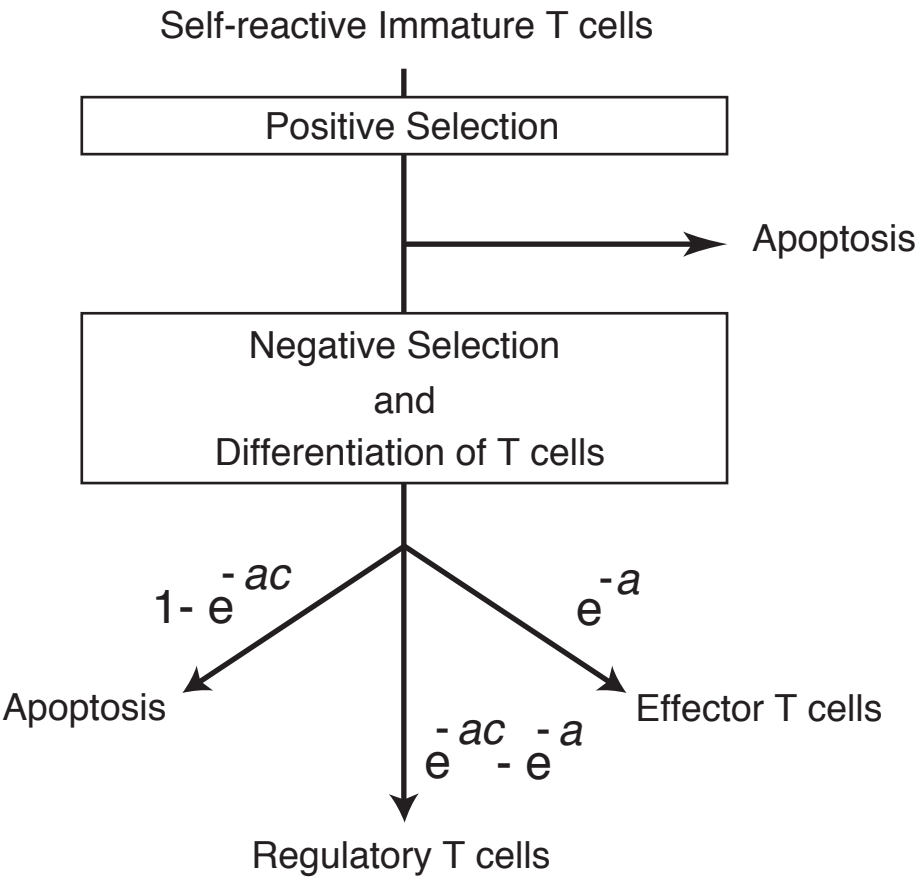


Figure 1. 5


Self-reactive Immature T cells
Positive Selection
Apoptosis
Negative Selection
and
Differentiation of T cells
1- e -ac
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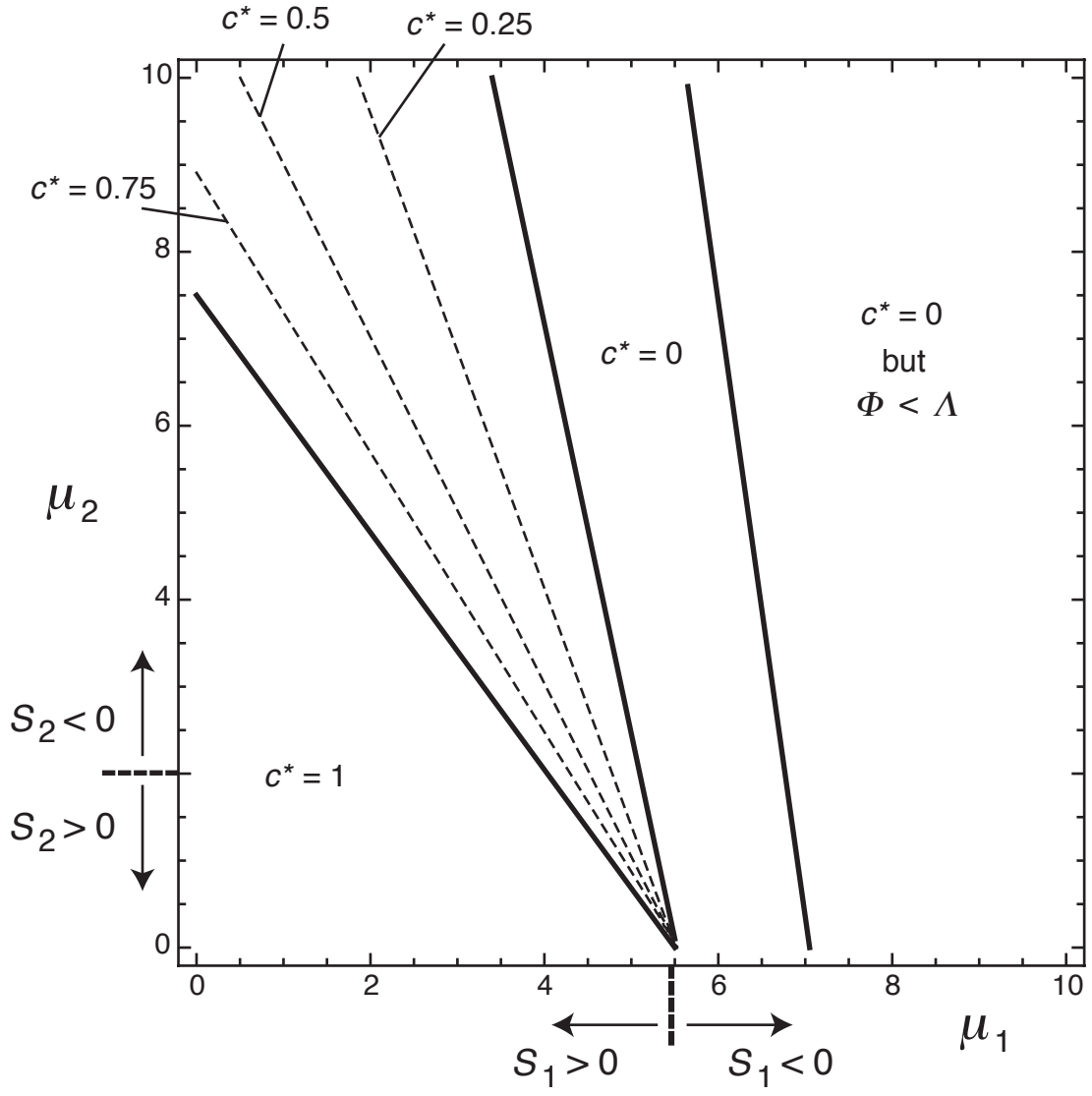
Apoptosis
Effector T cells
e -ac - e -a
Regulatory T cells

Figure 1. 6



Chapter 2

Optimal number of regulatory T cells

The study of this chapter was published in

Saeki, K., and Iwasa, Y., *Journal of Theoretical Biology*, **263**, 210-218 in 2010.

Introduction

I have developed a mathematical model discussing the condition in which having regulatory T cell is advantageous by considering the benefit of suppressing autoimmune diseases and the cost of jeopardizing the immune reactions to cope with foreign antigens. Analysis indicated that producing regulatory T cells is not advantageous if activated regulatory T cells suppress effector T cells at any location of a body (global suppression). In contrast it can be beneficial when a body is composed of many compartments and regulatory T cells suppress immune reactions only within the same compartment (localized suppression). One example of this localized suppression is the suppression only on the same antigen presenting cells (APCs) (Cobbold et al., 1996; Davies et al. 1996; Frasca et al., 1997; Wise et al., 1998).

Here a model of the suppression by regulatory T cells on an APC is studied, and the optimal number of regulatory T cells discussed by considering the benefit and harm of immune reactions, and the maintenance cost of regulatory T cells. The major assumption is thagt when a regulatory T cell recognize a self-epitope on an APC, it stays there and starts suppressing the activation of other naive T cells on the APC. The optimal number of regulatory T cells to maximize the benefit is analytically calculated, and I show the number depends on the amount of self-antigens, the severity of the autoimmunity, the abundance of pathogenic foreign antigens, and the spatial distribution of self-antigens in a body. I also discuss two different distributions of self-antigens, and two different types of regulation to produce regulatory T cells.

Model

The definition of fitness is the same as Eq.(1. 1), which is proportional to the abundance of self-reactive and non-self-reactive effector T cells. In addition, the maintenance cost of regulatory T cells are also included because I will discuss the optimal number of regulatory T cells. Producing regulatory T cells means that the immune system keeps additional amount

of T cells which is supposed to be removed due to their reactivity to self-antigens. Thus additional resources or apace to maintain those T cells are essential. Consequently, the fitness in this chapter consists of basic fitness without any T cells, the benefit of coping with foreign antigens provided by non-self-reactive effector T cells, the negative effect provided by self-reactive effector T cells, and the maintenance cost of regulatory T cells. The last three components in the fitness are affected by the number of regulatory T cells.

I focus on one possible mode of realizing the localized suppression of immune reactions, that is, the suppression of T cell activation is localized only on the same APC (Fig. 2. 1). APCs correct various antigens from the peripheral region of a body, and these antigens are digested into peptides which are presented on the cell surface (I call the peptide epitope). A naive T cell becomes activated when it recognizes its corresponding epitope on an APC. I assume that an activated conventional T cell leaves from a APC and explores to search for sites to work as an effector T cell. In contrast, when a regulatory T cell recognizes the peptide that it can recognize, then it stays on the same APC, and prevents other naive T cells from being activated. Let the number of epitopes per APC be k, and the fraction of self-epitopes be u. When epitopes presented on an APC follows their fraction, the fraction of APCs with i self-epitopes, A_i, follows a biomial distribution

$$A_i = \binom{k}{i} u^i (1-u)^{k-i} \qquad (2.1)$$

Let T_N be the number of non-self-reactive naive T cells, T_S be the number of self-reactive naive T cells, T_R be the number of regulatory T cells, and h be the encounter rate between an APC and a T cell, and ε be the mortality of APCs. The expected life time of an APC is $1/\varepsilon$, and the expected number of times for an APC to encounter with regulatory T cells during its life time is about hT_R/ε, which is assumed to be much greater than 1. By calculating the expected number of activated self-reactive and non-self-reactive naive T cells, fitness is expected as follows (the derivation is shown in Appendix)

$$\Phi \approx \Lambda + A_0 \frac{kh\nu T_N}{\varepsilon} - \frac{1}{T_R}\sum_{i=1}^{k} A_i \left[\mu T_S - \frac{(k-i)\nu T_N}{i} \right] - \gamma T_R \qquad (2.2)$$

where Λ is basic fitness without any T cells, ν is the benefit by a non-self-reactive effector T cell, μ is the harm of autoimmunity by a self-reactive effector T cell, and γ is the cost per T cell. The fitness can increase with the amount of regulatory T cells only when the sum in Eq.(2. 2) is positive. This condition can be rewritten as

$$\frac{\mu T_S}{\nu T_N} > \frac{1}{1-A_0}\sum_{i=1}^{k} \frac{k-i}{i} A_i \qquad (2.3)$$

The right hand side of (2. 3) is the function of the abundance of self-antigens, u, because A_i depends on u. A_i /(1- A_0) with i = 1, 2, 3, …, k, is a binomial distribution without zero term (normalized). Hence the mean of this truncated binomial distribution increases as the mean of the original binomial distribution u increases. In contrast, the mean of 1/i with this truncated binomial distribution should decrease with u. Consequently, the right hand side decreases with ku (Fig. 2. 1a) and inequality (2. 3) is more likely to be satisfied when the abundance of self-antigen u is large. The optimal number of regulatory T cells that maximize Eq.(2. 2), $\hat{T}_R$, is easy to be calculated,

$$\hat{T}_R = \sqrt{\frac{1}{\gamma}\sum_{i=1}^{k} A_i \left[\mu T_S - \frac{(k-i)\nu T_N}{i} \right]} \qquad (2.4)$$

Note that condition (2. 3) is equivalent to the positivity of the terms under the square root symbol. When the condition (2. 3) is violated, no regulatory T cells should be produced.

Parameter dependence

Abundance of self-antigens, u

Since the right hand side of (2. 3) decreases with the abundance of self-antigen, u, the condition to have regulatory T cells is more likely to hold for larger u (Fig. 2. 2a). The left hand side of (2. 3) must be larger than the curves in Fig. 2. 2a for having regulatory T cells to

be beneficial. Fig. 2. 2b indicates that the optimal number of regulatory T cells given by Eq.(2.4), which is an increasing function of u. In Fig. 2. 2b, $\hat{T}_R = 0$ corresponds to the situation in which condition (2. 3) is violated.

Parameters about T cell

As the severity of autoimmunity, μ, increases, producing regulatory T cells becomes more likely to be beneficial and the optimal number of regulatory T cells increases as illustrated by the curve with q = 1 in Fig. 2. 3a (q will be explained later). In contrast, as the benefit of coping with foreign antigens, ν, increases, producing regulatory T cells becomes less likely to be beneficial and also the optimal number of regulatory T cells decreases (Fig. 2. 3b, the curve with q = 1). If ν and μ are increased with the ratio μ / ν fixed, the condition to have regulatory T cells remains unchanged but the optimal number decreases.

Since T_S and μ appear in the fitness as their product μT_S, the dependence of the system on T_S is the same as that on μ. In a similar manner, T_N and ν appear in the fitness as their product νT_N. Hence, the horizontal axes in Fig. 2. 3a and b are chose as μT_S and νT_N respectively. If the number of self-reactive T cells increases, having regulatory T cells becomes more likely to be beneficial and the optimal number of regulatory T cells increases. The opposite result is obtained if the number of non-self-reactive T cells increases. The condition Eq.(2. 3) is independent of the maintenance cost of regulatory T cells, γ. However, once the condition holds, the optimal number of regulatory T cells given by Eq.(2. 4) decreases with γ (Fig. 2. 3c, the curve with q = 1).

Parameter about APC

Neither the condition for having regulatory T cells nor the optimal number of regulatory T cells depends on encounter efficiency, h. This result can be explained intuitively that high encounter efficiency promotes the useful immune reactions (i.e. encounters to naive T cells reactive to foreign antigens) as well as the harmful immune reactions (i.e. encounters to

self-reactive naive T cells) by the same factor.

Fig. 2. 2a illustrates the threshold level of $\mu T_S/\nu T_N$ for having regulatory T cells to be beneficial given by condition (2. 3), and Fig. 2. 2b illustrates the optimal number of regulatory T cells to produce. As k increases, the right hand side of condition (2. 3) increases if the fraction of self-antigens u is small, but it looks like the same for very large u. The optimal number of regulatory T cells increases with k for large u in Fig. 2. 2b, but in general the dependence on k for small u is quite complicated.

Localization of self-antigen in a body

I have considered the situation in which self-antigens would distribute evenly over the whole body. More likely is the situation in which self-antigens exist in a small portion of a body. Here I consider the effect of spatially aggregated distribution of self-antigens on the optimal number of regulatory T cells. Suppose that some fraction of APCs come from the sections in which the concentration of self-antigen is high, and denote the fraction of the sections by q. The 1- q fraction of a body contains no self-antigens to which self-reactive T cells are produced. In this case, the number of activated self-reactive T cells become smaller than in Eq.(2. 2), and fitness is expressed as,

$$\Phi \approx \Lambda + \left(1 - q + qA_0(u/q)\right)\frac{kh\nu T_N}{\varepsilon} - \frac{q}{T_R}\sum_{i=1}^{k} A_i(u/q)\left[\mu T_S - \frac{(k-i)\nu T_N}{i}\right] - \gamma T_R \quad (2.5)$$

The local abundance of self-antigen is u/q, which is enhanced for small q, because the total fraction of self-antigens in a body is fixed. To show this dependence explicitly, I used the expression $A_i(u/q)$. Since the local abundance must satisfy $u/q \le 1$, relationship $u \le q \le 1$ must be satisfied. The optimal number of regulatory T cells is

$$\hat{T}_R = \sqrt{\frac{q}{\gamma}\sum_{i=1}^{k} A_i(u/q)\left[\mu T_S - \frac{(k-i)\nu T_N}{i}\right]} \quad (2.6)$$

Eq.(2. 6) indicates that the maintenance cost becomes enhanced by the fact of $1/q$, and it

makes the optimal number smaller. On the other hand, it also depends on u which should be changed as u/q.

The dependence of the optimal number of regulatory T cells, $\hat{T}_R$, on q and u is checked (Fig. 2. 4). When u is fixed at low value, $\hat{T}_R$ increases with q for relatively small q, but it has a peak and then decreases with q for large q. When u is fixed high, $\hat{T}_R$ increases with q and there is no peak in $u \le q \le 1$ (Fig. 2. 4a). This result can be explained as follows: When q is small, the maintenance cost of regulatory T cells is large and the optimal number of regulatory T cell becomes small. In contrast when q is large, the local abundance of self-antigens becomes small and again the optimal number becomes small. The optimal number of regulatory T cells becomes a maximum for an intermediate value of q. On the other hand, Fig. 2. 4b shows how $\hat{T}_R$ depends on the local abundance of self-antigen, u/q, with fixed q. The local abundance is important in determining whether regulatory T cells should be produced or not, and $\hat{T}_R$ becomes smaller as q decreases with local abundance kept constant.

Multiple types of self-antigens with segregated distribution

For simplicity, I focus on the case of just two types of self-antigens distinguished by suffix m. Suppose the frequency of type-m self-antigen by u_m, the number of naive and regulatory T cells reactive to type-m self-antigen by T_{Sm} and T_{Rm} respectively. Let μ_m be the severity of attacking type-m self-antigen. Let $A_{i,j}$ be the fraction of APCs that have exactly i type-1 and exactly j type-2 self-antigens. According to the Appendix, fitness is expressed as follows when ε is sufficiently small

$$\Phi \approx \Lambda + A_{0,0}\frac{kh\nu T_N}{\varepsilon} + \sum A_{i,j}\left[\frac{(k-i-j)\nu T_N}{iT_{R1}+jT_{R2}} - \frac{i\mu_1 T_{S1}+j\mu_2 T_{S2}}{iT_{R1}+jT_{R2}}\right] - \gamma\left(T_{R1}+T_{R2}\right) \quad (2.7)$$

I ask what are the optimal values of T_{R1} and T_{R2} that maximize Eq.(2. 7). The fitness function Eq.(2. 7) assumes that both self-antigens are distributed evenly over a body, but it would be more likely that self-antigens are concentrated to a small portion of a body. In the following, I consider the two simple cases in which the distributions of the two types of self-antigens are concentrated.

At first, let consider the case in which the two types of self-antigens are concentrated in different subsections of a body and they are not colocalized at all. In this case, two terms including T_{R1} and T_{R2} are separated and Eq.(2. 7) becomes

$$\begin{aligned}\Phi \approx \Lambda &+ \left(1-2q+2qA_{0,0}(\frac{u_1}{q_1},\frac{u_2}{q_2})\right)\frac{kh\nu T_N}{\varepsilon} - \frac{q}{T_{R1}}\sum_{i=1}^{k} A_{i,0}(\frac{u_1}{q_1},\frac{u_2}{q_2})\left[\mu_1 T_{S1} - \frac{(k-i)\nu T_N}{i}\right] \\ &- \frac{q}{T_{R2}}\sum_{j=1}^{k} A_{0,j}(\frac{u_1}{q_1},\frac{u_2}{q_2})\left[\mu_2 T_{S2} - \frac{(k-j)\nu T_N}{j}\right] - \gamma\left(T_{R1}+T_{R2}\right)\end{aligned} \tag{2. 8}$$

where parameter q is same between two types ($0 \le q \le 0.5$). According to Eq.(2. 8), the optimal T_{R1} and T_{R2} that maximize Φ are the same as the case with only one type of self-antigen. Yherefore the condition for the positivity of the optimal T_{R1} and T_{R2} is condition (2. 3) for each type (m = 1, 2), and the optimal number of regulatory T cells is expressed as Eq.(2. 4), in which symbols have suffix

$$\hat{T}_{R1} = \sqrt{\frac{q}{\gamma}\sum_{i=1}^{k} A_i(u_1/q)\left[\mu_1 T_{S1} - \frac{(k-i)\nu T_{N1}}{i}\right]} \tag{2. 9a}$$

$$\hat{T}_{R2} = \sqrt{\frac{q}{\gamma}\sum_{i=1}^{k} A_i(u_2/q)\left[\mu_2 T_{S2} - \frac{(k-i)\nu T_{N2}}{i}\right]} \tag{2. 9b}$$

All the arguments on the parameter dependence of the optimal number derived in the last section hold for Eq.(2. 9).

When immature T cells cannot tell the type of self-antigen

I have not discussed on the mechanism controlling the number of regulatory T cells, however the mechanism may cause an important constraint in producing regulatory T cells. In the previous chapter, I assumed that the differentiation of regulatory t cells is made based on

their recognition of antigens in the thymus, and that all regulatory T cells are reactive to self-antigens. If immature T cells do not interact with antigens, they could be expected that they are not reactive to self-antigens, hence they should become naive T cells. In contrast if immature T cells interact with antigens in the thymus, they should become regulatory T cells as they know that they can react to self-antigens. By adjusting the probability of cell death per interaction, I can control the number of regulatory T cells to produce (Chapter 1, Inducing selection and differentiation at the same time). This mechanism could be common among different types of self-antigens if immature T cells cannot detect the type of self-antigen with which they are interacting. Then, the same number of regulatory T cells might be produced.

I next consider the situation with the constraint of $T_{R1} = T_{R2} = T_{Rc}$, and Eq.(2. 7) is rewritten as

$$\Phi \approx \Lambda + A_{0,0}\frac{kh\nu T_N}{\varepsilon} + \frac{1}{T_{Rc}}\sum_{0<i+j\le k} A_{i,j}\left[\frac{(k-i-j)\nu T_N}{i+j} - \frac{i\mu_1 T_{S1} + j\mu_2 T_{S2}}{i+j}\right] - 2\gamma T_{Rc} \quad (2.10)$$

If two types of self-antigens are completely segregated in a body, the optimal abundance of regulatory T cells is described as

$$\hat{T}_{Rc} = \sqrt{\frac{q}{2\gamma}\left[\begin{array}{l}\displaystyle\sum_{i=1}^{k} A_{i,0}\left(\frac{u_1}{q_1},\frac{u_2}{q_2}\right)\left(\mu_1 T_{S1} - \frac{(k-i)\nu T_N}{i}\right) \\ \displaystyle+\sum_{j=1}^{k} A_{0,j}\left(\frac{u_1}{q_1},\frac{u_2}{q_2}\right)\left(\mu_2 T_{S2} - \frac{(k-j)\nu T_N}{j}\right)\end{array}\right]} \quad (2.11)$$

When the optimal number of regulatory T cells differs between two types ($\hat{T}_{R1} \neq \hat{T}_{R2}$), T_{R1}, T_{R2}, and T_{Rc} are plotted for different abundance of type-1 self-antigen, u_1 (Fig. 2. 5). Without the limitation, the optimal number of regulatory T cell for the more abundant type is larger than the other. However $\hat{T}_{Rc}$ exists between $\hat{T}_{R1}$ and $\hat{T}_{R2}$, and satisfies

$$\hat{T}_{Rc} = \sqrt{\left(\hat{T}_{R1}^2 + \hat{T}_{R2}^2\right)/2} \quad (2.12)$$

if both $\hat{T}_{R1}$ and $\hat{T}_{R2}$ are positive.

Multiple self-antigens that are colocalized

Next let consider the situation in which two types of self-antigens are strongly colocalized in the same portion of a body. A typical case is that two antigens are either two different aspects of the same proteins or of two proteins co-occurring in the same organ. Specifically I assume that there is a fraction q of a body in which two types of self-antigens exist whilst neither type of self-antigen exists in the rest. Then, Eq.(2. 7) is rewritten as

$$\begin{aligned}\Phi \approx \Lambda &+ \left(1-q+qA_{0,0}\left(\frac{u_1}{q},\frac{u_2}{q}\right)\right)\frac{kh\nu T_N}{\varepsilon} \\ &+q\sum_{0<i+j\le k} A_{i,j}\left(\frac{u_1}{q},\frac{u_2}{q}\right)\left[\frac{(k-i-j)\nu T_N}{iT_{R1}+jT_{R2}}-\frac{i\mu_1 T_{S1}+j\mu_2 T_{S2}}{iT_{R1}+jT_{R2}}\right]-\gamma(T_{R1}+T_{R2})\end{aligned} \tag{2. 13}$$

The difference between this case and the segregated case studied in the last section illustrates the importance of spatial distribution. In this section, I first consider the case in which the number of regulatory T cells for type-1 and type-2 can be regulated separately. Then later discuss the case in which their abundances are constrained to be the same.

When two types of regulatory T cells are regulated separately

The optimal strategy, a pair of T_{R1} and T_{R2} for different abundance of type-1 self-antigen, u_1 that maximizes Eq. (2. 13), was obtained numerically. Fig. 6a illustrates the dependence of $\hat{T}_{R1}$ or $\hat{T}_{R2}$ on the risk of foreign antigens νT_N, and is divided into three regions. If two types of self-antigens are co-localized, regulatory T cells for each type should be produced at equal abundance for a small value of νT_N (labeled as I in Fig. 6a). For larger νT_N, the asymmetric production of regulatory T cells of two types is the optimal, to produce one of the two types of regulatory T cells is better than to produce both types (labeled as II). For very large νT_N, no regulatory T cells should be produced (labeled as III). According to this figure, the optimal number of regulatory T cells when two types of self-antigens are

colocalized is smaller than the optimal when two self-antigens are segregated for small νT_N. As νT_N increases, the optimal number becomes greater when colocalized than when segregated. There is a parameter region in which no regulatory T cells should be produced if self-antigens are segregated, but some regulatory T cells should be produced if they are colocalized. The region in which the suppression of non-self-reactive T cells occurs is wider in the segregated case than in the colocalized case. Thus, the disadvantage caused by regulatory T cells is heavier than the benefit given by them in former case but it is smaller in the latter case.

Fig. 2. 6b indicates the optimal number of regulatory T cells for each type (the curve labeled as $\hat{T}_{Rc}$ will be explained later). Horizontal axis is the abundance of type-1 self-antigen, u_1, and the abundance of type-2 self-antigen, u_2, is fixed. The optimal strategy is to produce one type of regulatory T cell that is reactive to the more abundant type. As a consequence, for small u_1, a large number of type-2 regulatory T cells should be produced whilst for large u_1, a large number of type-1 regulatory T cells should be produced. Note that the switch occurs when $u_1 = u_2$. It is illuminating to compare Fig. 2. 6b when two self-antigens are colocalized with Fig. 2. 5 when they are segregated. In Fig. 2. 5, as the abundance of type-1 self-antigen increases, the optimal number of regulatory T cells for type-1 increases, but the number of regulatory T cells for type-2 self-antigen is kept constant.

When two types of regulatory T cell numbers are constrained to be equal

If a host is restricted to produce the same number of regulatory T cells for different types of self-antigens and if the self-antigens are colocalized, the optimal number of regulatory T cells to produce can be calculated numerically. In the region I of Fig. 2. 7a, the number of regulatory T cells for each type is the same as when they are not constrained because the optimal when free to chose happens to be producing two types equally. However, for larger νT_N (region labeled as II), in which the optimal when free to choose is producing only one type and none of the other type, the optimal solution with the constraint is different.

Interestingly, Fig. 2. 7b shows that the total number of regulatory T cells is larger when the number of regulatory T cells of different types can be chosen separately than when they are constrained to be equal. In Fig. 2. 6b, the result when the regulatory T cells of two types are constrained to be equal is indicated by a curve labeled as $\hat{T}_{Rc}$. It is in between the optimal number of regulatory T cells of two types when they can be produced at different levels as in the case of segregated self-antigens.

Discussion

I considered the optimal number of regulatory T cells to produce with the localized suppression on antigen presenting cells. The basic assumption is that, when a regulatory T cell is activated by the self-antigen, it stays on the same APC and suppresses the activation of other T cells that come to the APC. When there is a single type of self-antigen distributed uniformly in a body, having regulatory T cells is likely to be beneficial when the abundance of self-antigens and the severity of attacking self-antigens are high, the number of naive T cells reactive to self-antigens is large, and the advantage of attacking foreign antigens is low. Under the same condition, the optimal number of regulatory T cells tends to be large.

The spatial distribution of self-antigens greatly affects both the condition for producing regulatory T cells to be advantageous and the optimal number of regulatory T cells. When self-antigens are confined in a small fraction of a body, the local abundance of self-antigens is important in determining the optimal number of regulatory T cells. If the total abundance of the self-antigens in the section is controlled, the optimal number of regulatory T cells still depends on the spatial distribution in quite a complex manner.

When there are multiple types of self-antigens, whether their distributions are overlapped or not, also have an impact. If they are perfectly segregated and if the abundance for different types of regulatory T cells can be regulated independently, the optimal number of regulatory T cells for each type is exactly the same as the one calculated for a single type

of self-antigen. However the result is very different when the two self-antigens are spatially concentrated in the same section of a body. In this case, reacting to one type of self-antigen would also work for suppressing the immune reactions to the other type. As a consequence, producing only one of the two types of regulatory T cells can be better than producing both types for some parameters. The specially concentrated distribution of self-antigens in the analyses can be interpreted as the difference in the life-stages in which self-antigens appear. For example if one type of self-antigen appear only on youth whilst the second type appear only in old ages, and if their age of appearance is not overlapped, they are two self-antigens of segregated distributions in the present model.

I did not consider the mechanisms controlling the number of regulatory T cells, but some possibilities have been discussed (Modigliani et al., 1996; Jordan et al., 2001; Van Santen et al., 2004). Several experiments support the idea that interactions with self-antigen in the thymus enhance the differentiation of immature T cells to regulatory T cells (e.g., Hsieh et al., 2006; Jordan et al., 2001). Choosing the mortality per interaction during the training period in the thymus is equivalent to regulating the number of regulatory T cells. This mechanism should give constraints on the number of regulatory T cells to produce: First, if a self-antigen is very rare and difficult to encounter in the thymus during the training period, the number of regulatory T cells reactive to it will be zero or very small. Second, if there are multiple types of self-antigens, and if the number of immature T cells reactive to each self-antigen and the efficiency of interaction in the thymus are equal among self-antigens, then the same number of regulatory T cells will be produced for different types of self-antigens. These constraints given by the mechanism of the differentiation of regulatory T cells could explain the recent experimental observation of transgenic made by Bautista et al. (2009) who suggest that the total number of regulatory T cells is bounded.

Although I discussed the case with one or two types of self-antigens, extending the formalism enables to discuss the optimal number of regulatory T cells for each of n types when they are regulated separately. Suppose for the moment that these self-antigens are

strongly localized in different parts of a body or in different life stages. Then, their optimal abundances are calculated from Eq.(2. 4). If their abundances are either high or mildly low in the training period, the optimal number of regulatory T cells could be produced. If instead the numbers of regulatory T cells for different types of self-antigens are constrained to be equal due to the informational constraint during the training period, the number of regulatory T cells to produce would be either lower or higher than the optimal value of Eq.(2. 4), which is different among the type of self-antigen.

Here I focused on naturally occurring regulatory T cells, which develop in the thymus upon interactions with self-antigens. However, there exist adaptive (or induced) regulatory T cells as well (Vukmanovic-Stejic et al. 2006). These cells may contrite to the tolerance to self-antigens that are too rare to be produced in the thymus. The role of these induced regulatory T cells in the regulation of locally segregated self-antigens should be an important theme of future theoretical study. The results all depend on different rate parameters, the mode of training, the availability of the information to the immature T cells, relative abundance of different types of self-antigens and many others, which are currently unknown. To understand the functioning of the immune system including regulatory T cells, it is essential to reveal more of these quantitative aspects of the biology as well as the molecular mechanism of immune system regulation.

Appendix: Derivation of fitness

Single type of self-antigen

If an APC does not have any self-antigens ($i = 0$), the expected number of non-self-reactive T cells that are activated is

$$\int_0^\infty te^{-\varepsilon t} khT_N \varepsilon \, dt = \frac{khT_N}{\varepsilon} \tag{2. 14}$$

where ε is the mortality of an APC. When $i > 0$, the expected number of non-self-reactive T cells that are activated is

$$\int_0^\infty te^{-(ihT_R+\varepsilon)t}(k-i)hT_N(ihT_R+\varepsilon)\,dt = \frac{(k-i)hT_N}{ihT_R+\varepsilon} \tag{2. 15}$$

In contrast, the expected number of self-reactive T cells to be activated is

$$\int_0^\infty te^{-(ihT_R+\varepsilon)t} ihT_S(ihT_R+\varepsilon)\,dt = \frac{ihT_S}{ihT_R+\varepsilon} \tag{2. 16}$$

Hence the fitness is

$$\Phi = \Lambda + A_0 \frac{kh\nu T_N}{\varepsilon} + \sum_{i=1}^{k} A_i \left[\frac{(k-i)h\nu T_N}{ihT_R+\varepsilon} - \frac{ih\mu T_S}{ihT_R+\varepsilon} \right] \tag{2. 17}$$

If I assume $hT_R >> \varepsilon$, Eq.(2.) is approximated as

$$\Phi \approx \Lambda + A_0 \frac{kh\nu T_N}{\varepsilon} - \frac{1}{T_R} \sum_{i=1}^{k} A_i \left[\mu T_S - \frac{(k-i)\nu T_N}{i} \right] \tag{2. 18}$$

Including the maintenance cost of regulatory T cells, Eq. (2. 2) is obtained.

Multiple types of self-antigens

If an APC have number i of type-1 self-antigen and number j of type-2 self-antigen, the expected number of non-self-reactive T cells that are activated is

$$\frac{h(k-i-j)T_N}{h(iT_{R1}+jT_{R2})+\varepsilon}$$

The expected number of self reactive T cell that are activated is

$$\frac{h(iT_{S1}+jT_{S2})}{h(iT_{R1}+jT_{R2})+\varepsilon}$$

Let $A_{i,j}$ be the fraction of APC that have exactly i type-1 self-antigen and exactly j type-2

self-antigen. Then fitness is expressed as

$$\Phi = \Lambda + \sum_{i=0}^{k} \sum_{j=0}^{k-i} A_{i,j} \left[\frac{h(k-i-j)\nu T_N}{h(iT_{R1} + jT_{R2}) + \varepsilon} - \frac{h(i\mu_1 T_{S1} + j\mu_2 T_{S2})}{h(iT_{R1} + jT_{R2}) + \varepsilon} \right] - \gamma(T_{R1} + T_{R2}) \qquad (2.19)$$

When ε is sufficiently small, Eq.(2. 7) is obtained.

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Figure Legends

Figure 2. 1 Basic scheme of our model. Each antigen presenting cell (APC) has k epitopes, which can be self or non-self. If a regulatory T cell recognizes its own specific self-epitope on an APC, all the activations of naive T cells on the same APC are prevented regardless of their antigen specificity. However, the regulatory T cell cannot prevent the activation on different APCs.

Figure 2. 2 Dependence of important values on u in the simplest model. Different curves correspond to different values of k. (a) Right hand side of Eq.(2. 3). (b) Optimal number of regulatory T cells. Other parameters are: $\mu T_S = 8, \nu T_N = 1, \gamma = 1$.

Figure 2. 3 Parameter dependences of the optimal number of regulatory T cells. Horizontal axes are: (a) μT_S, (b) νT_N, (c) γ. Self-antigens are distributed in q of all the sections in a body. Other parameters are: $k = 10, u = 0.1, \mu T_S = 8, \nu T_N = 1, \gamma = 1$.

Figure 2. 4 Optimal number of regulatory T cells when self-antigens are restricted in a fraction q of the whole body. (a) Dependence on q when u is fixed. (b) Dependence on the local abundance of self-antigens u/q when q is fixed. Note that $u \le q \le 1$ should be satisfied. Other parameters are: $k = 10, \mu T_S = 8, \nu T_N = 1, \gamma = 1$.

Figure 2. 5 Optimal number of regulatory T cells when there are two types of self-antigens and they are completely segregated. The horizontal axis is the fraction of type 1 self-antigen, u_1. Curves labeled $\hat{T}_{R1}$ and $\hat{T}_{R2}$: when the number of regulatory T cells reactive to each type can be adjusted separately (calculated by Eq.(2. 9)). Curve labeled as $\hat{T}_{Rc}$: when the numbers of regulatory T cells for two types are constrained to be equal (calculated by Eq.(2. 11)). Other parameters are: $k = 10, q = 0.3, u_2 = 0.1, \mu_1 T_{S1} = \mu_2 T_{S2} = 8,$

$\nu T_N = 1, \gamma = 1.$

Figure 2. 6 Optimal number of regulatory T cells for each type of self-antigen ($\hat{T}_{R1}$ or $\hat{T}_{R2}$) is obtained numerically. (a) The dependence on the risk of foreign antigens νT_N. The optimal strategy when two types of self-antigens are colocalized (real line) has three regions, producing two types of regulatory T cells at equal abundance (labeled as I), producing only one of the two types (labeled as II), producing no regulatory T cells (labeled as III). The dashed line, which indicates the optimal when two types of self-antigens are segregated, is calculated by Eq.(2. 9). Parameters are $u_1 = u_2 = 0.1$. (b) Horizontal axis is the abundance of type-1 self-antigens u_1, whilst that of type-2 antigens remains fixed by $u_2 = 0.1$ and $\nu T_N = 1$. Curves labeled as $\hat{T}_{R1}$ and $\hat{T}_{R2}$ are for the optimal number of regulatory T cells for each. The curve labeled as T_{Rc} is the optimal number when regulatory T cells for two types are constrained to be equal. Other parameters are: $k = 10, q = 0.3, \mu_1 T_{S1} = \mu_2 T_{S2} = 8, \gamma = 1.$

Figure 2. 7 Optimal number of regulatory T cells when two types of self-antigens are co-localized. Horizontal axis is the risk of foreign antigens, νT_N. (a) Dashed line indicates the optimal strategy when two types of regulatory T cells are constrained to be equal ($\hat{T}_{Rc}$), and real line indicates the optimal when there is no constraint ($\hat{T}_{R1}$ or $\hat{T}_{R2}$). (b) Total number of regulatory T cells with the constraint ($2\hat{T}_{Rc}$, dashed line), and without it ($\hat{T}_{R1} + \hat{T}_{R2}$, real line). The definitions of three regions are the same in Fig. 6a, and $\hat{T}_{R1} = \hat{T}_{R2} = \hat{T}_{Rc}$ is satisfied in region I and III. Other parameters are: $k = 10, q = 0.3, u_1 = u_2 = 0.1, \mu_1 T_{S1} = \mu_2 T_{S2} = 8, \gamma = 1.$

Figure 2. 1

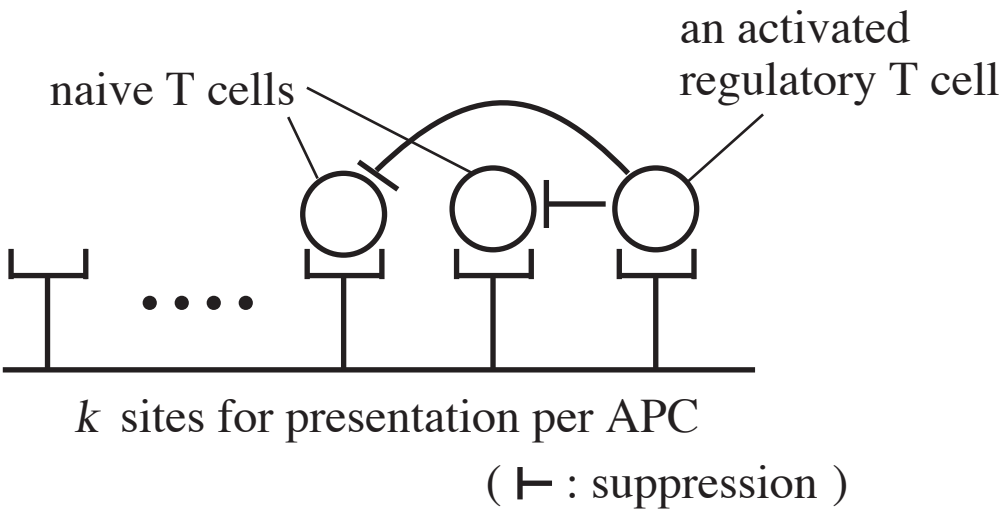

an activated
regulatory T cell
naive T cells
k sites for presentation per APC
(⊢ : suppression)

Figure 2. 2

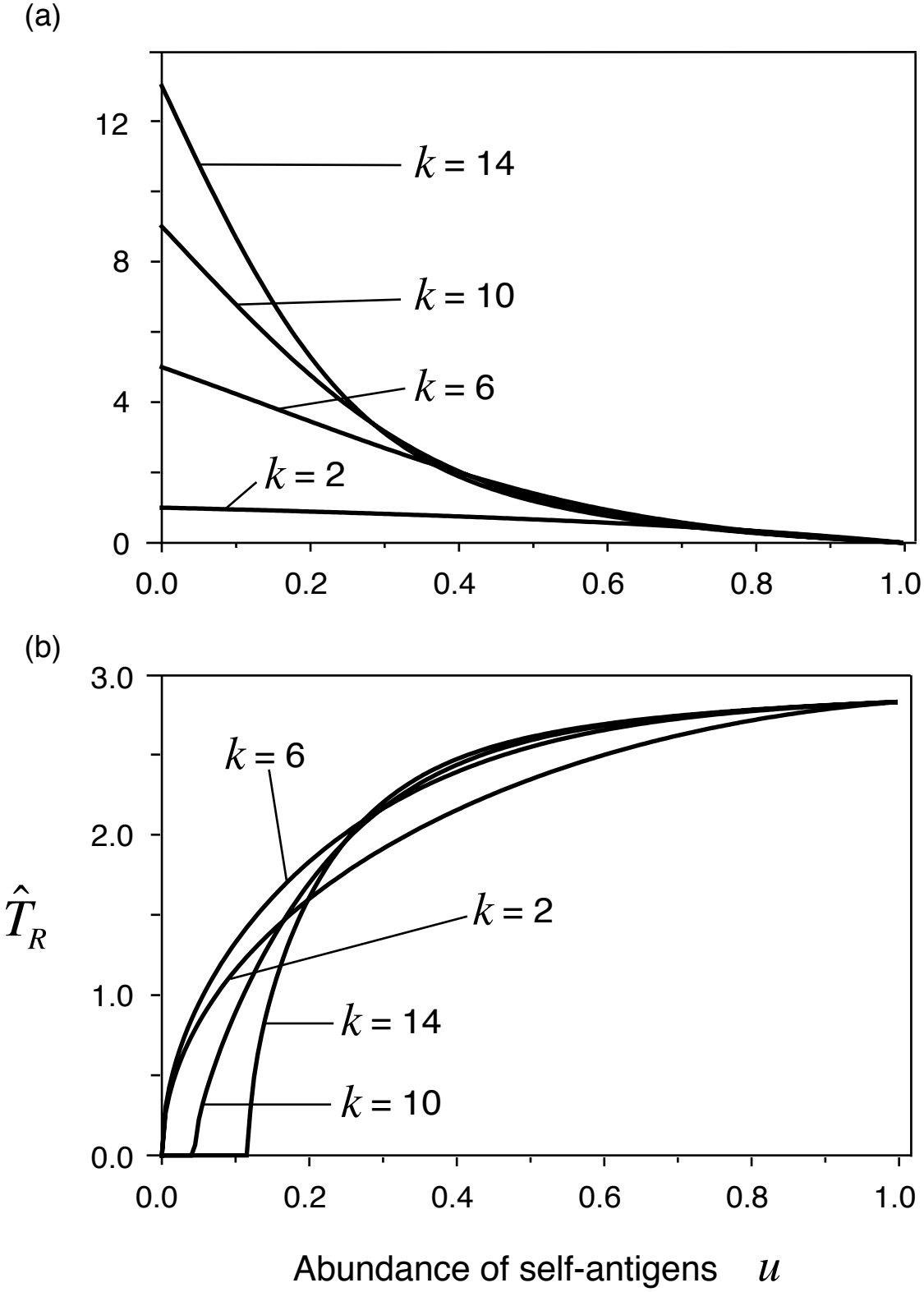

(a)
12
8
4
0
0.0
0.2
0.4
0.6
0.8
1.0
k = 14
k = 10
k = 6
k = 2
(b)
3.0
2.0
1.0
0.0
$\hat{T}_R$
k = 6
k = 2
k = 14
k = 10
Abundance of self-antigens u

Figure 2. 3

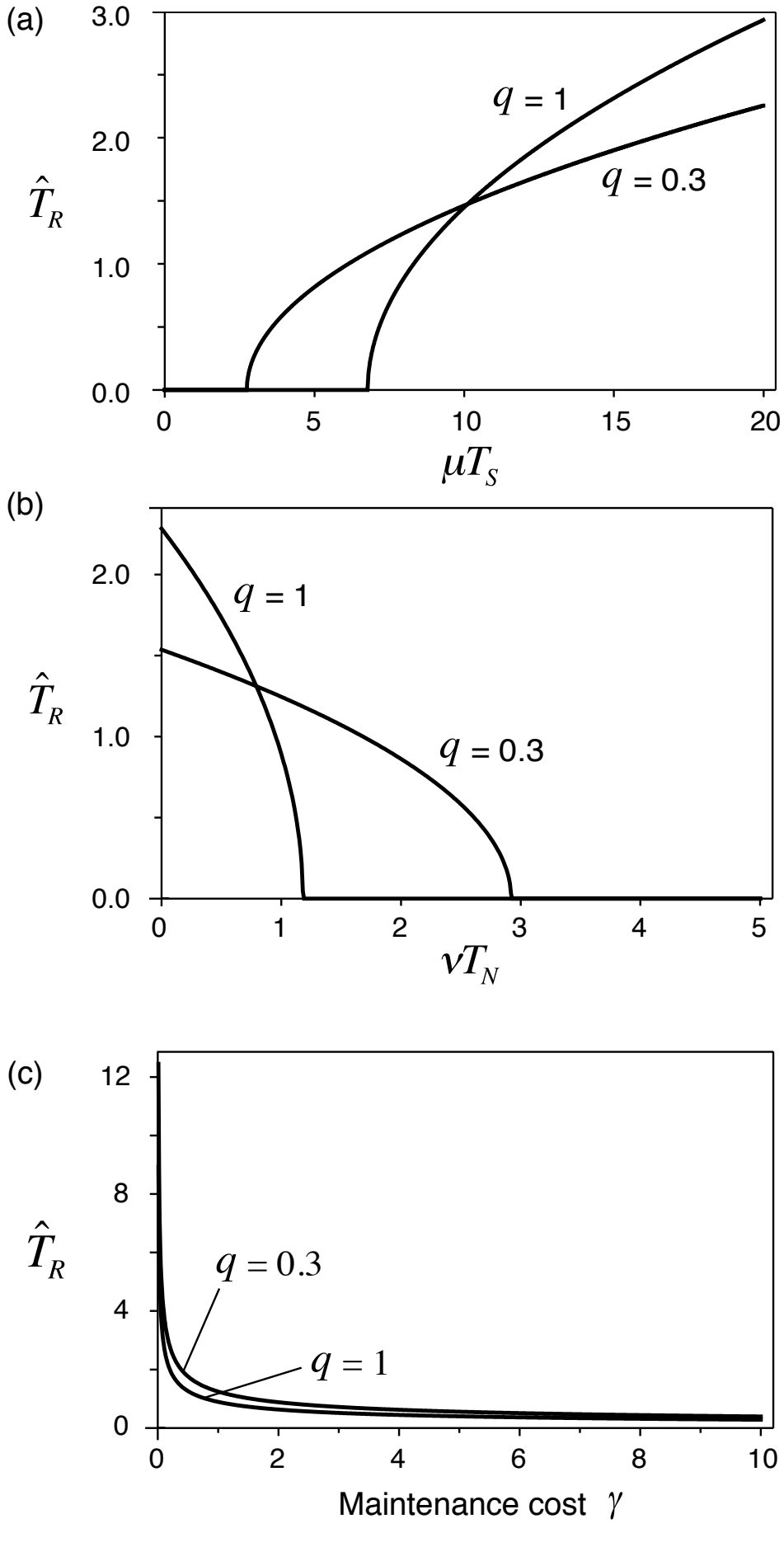

(a)
3.0
2.0
1.0
0.0
$\hat{T}_R$
$q = 1$
$q = 0.3$
0
5
10
15
20
μT_S
(b)
2.0
1.0
0.0
$\hat{T}_R$
$q = 1$
$q = 0.3$
0
1
2
3
4
5
νT_N
(c)
12
8
4
0
$\hat{T}_R$
$q = 0.3$
$q = 1$
0
2
4
6
8
10
Maintenance cost γ

Figure 2. 4

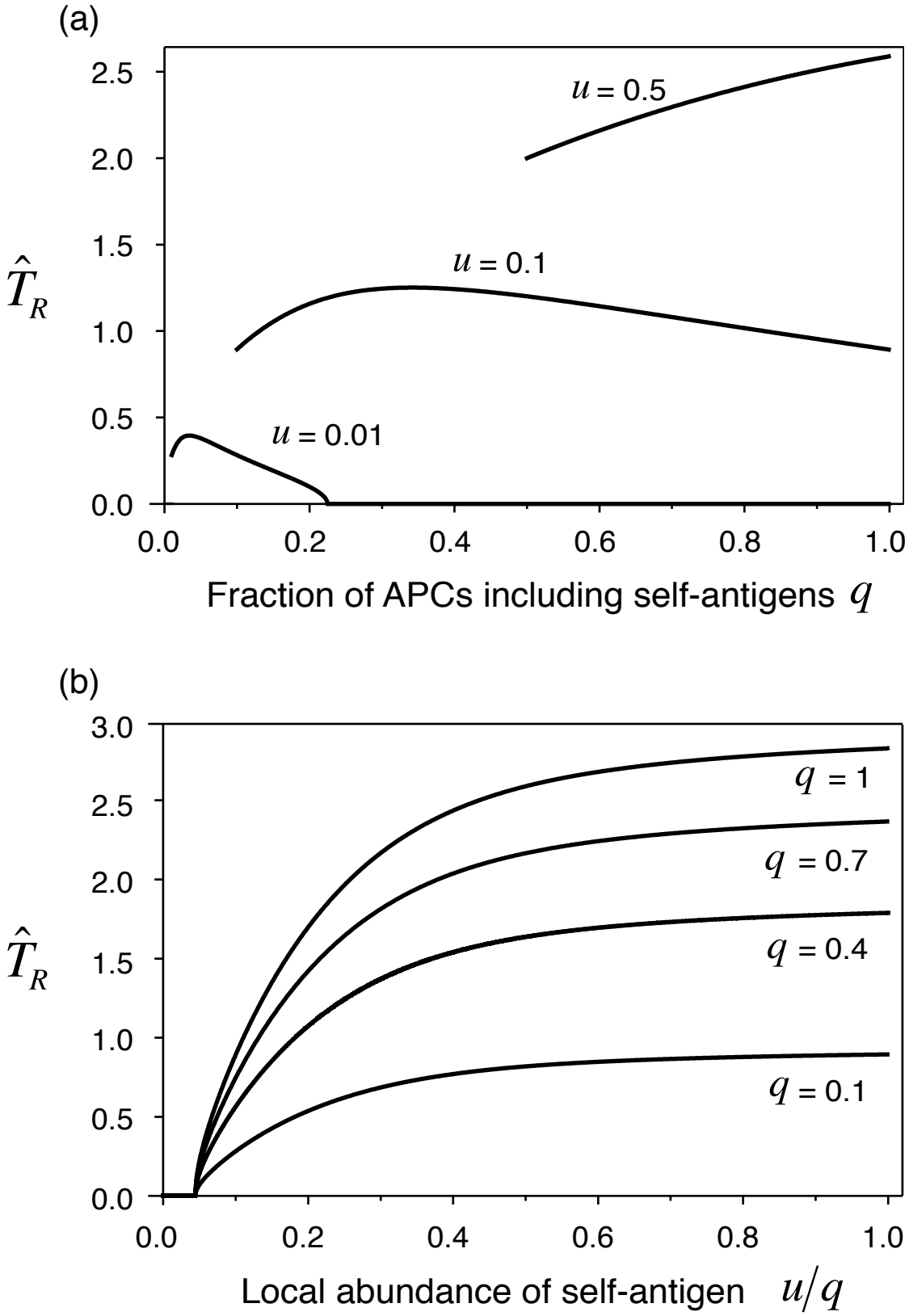

(a)
2.5
2.0
1.5
1.0
0.5
0.0
$\hat{T}_R$
u = 0.5
u = 0.1
u = 0.01
0.0
0.2
0.4
0.6
0.8
1.0
Fraction of APCs including self-antigens q
(b)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
$\hat{T}_R$
q = 1
q = 0.7
q = 0.4
q = 0.1
0.0
0.2
0.4
0.6
0.8
1.0
Local abundance of self-antigen u/q

Figure 2. 5

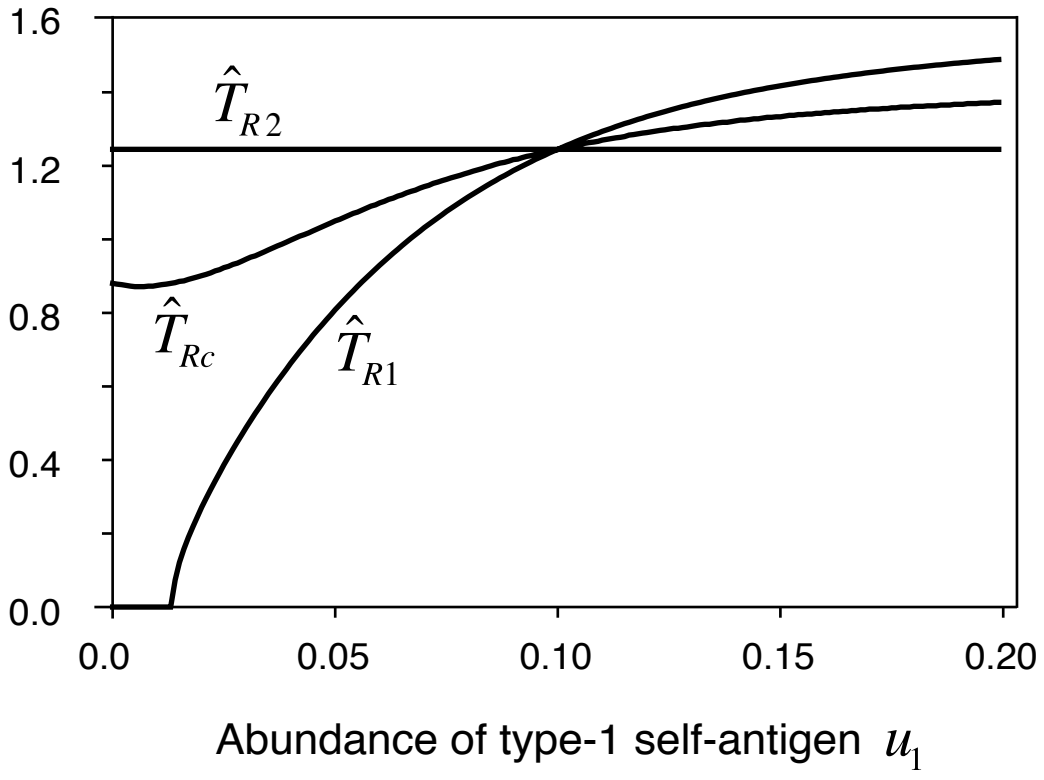

1.6
1.2
0.8
0.4
0.0
0.0
0.05
0.10
0.15
0.20
$\hat{T}_{R2}$
$\hat{T}_{Rc}$
$\hat{T}_{R1}$
Abundance of type-1 self-antigen u_1

Figure 2. 6

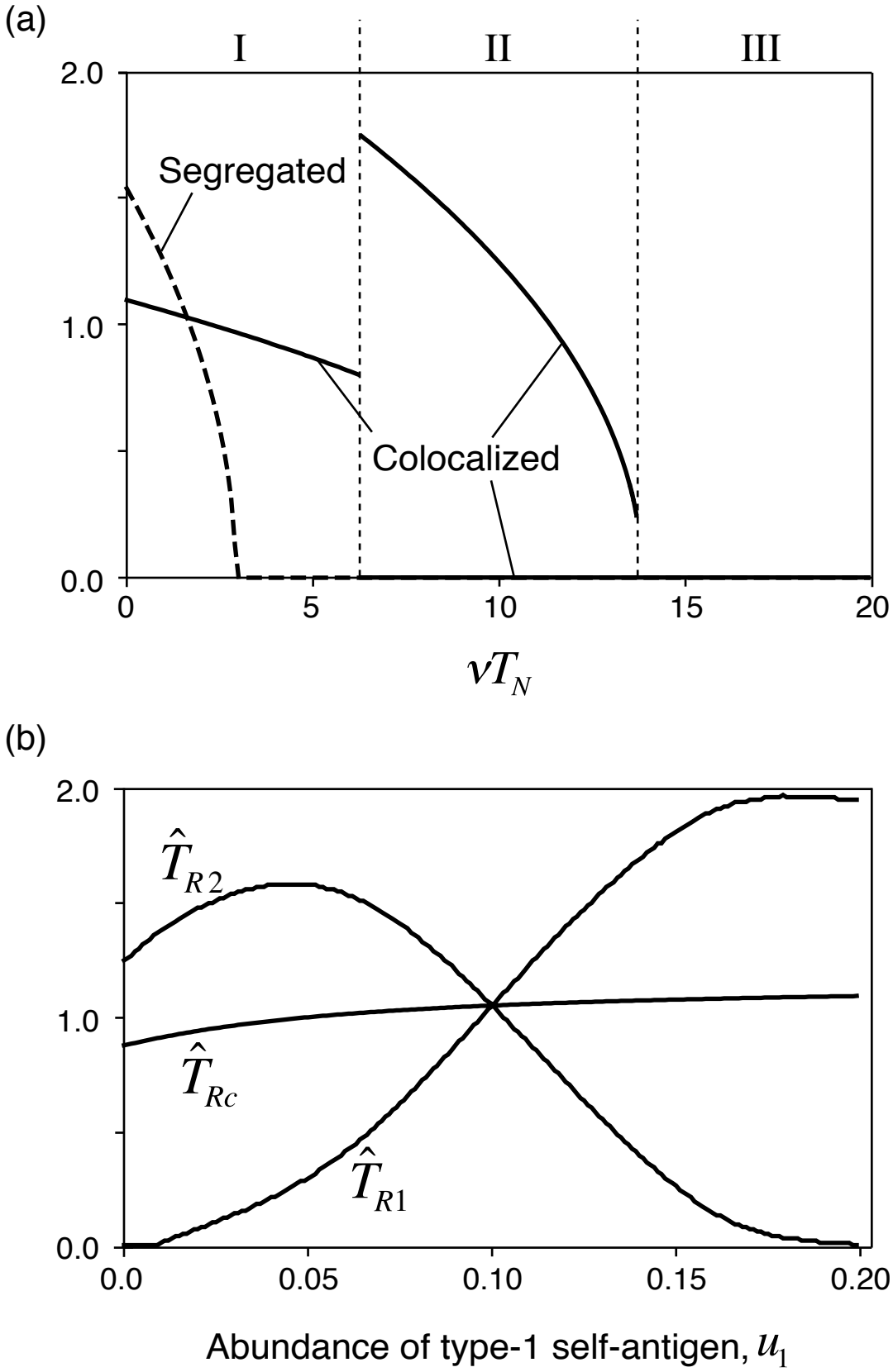
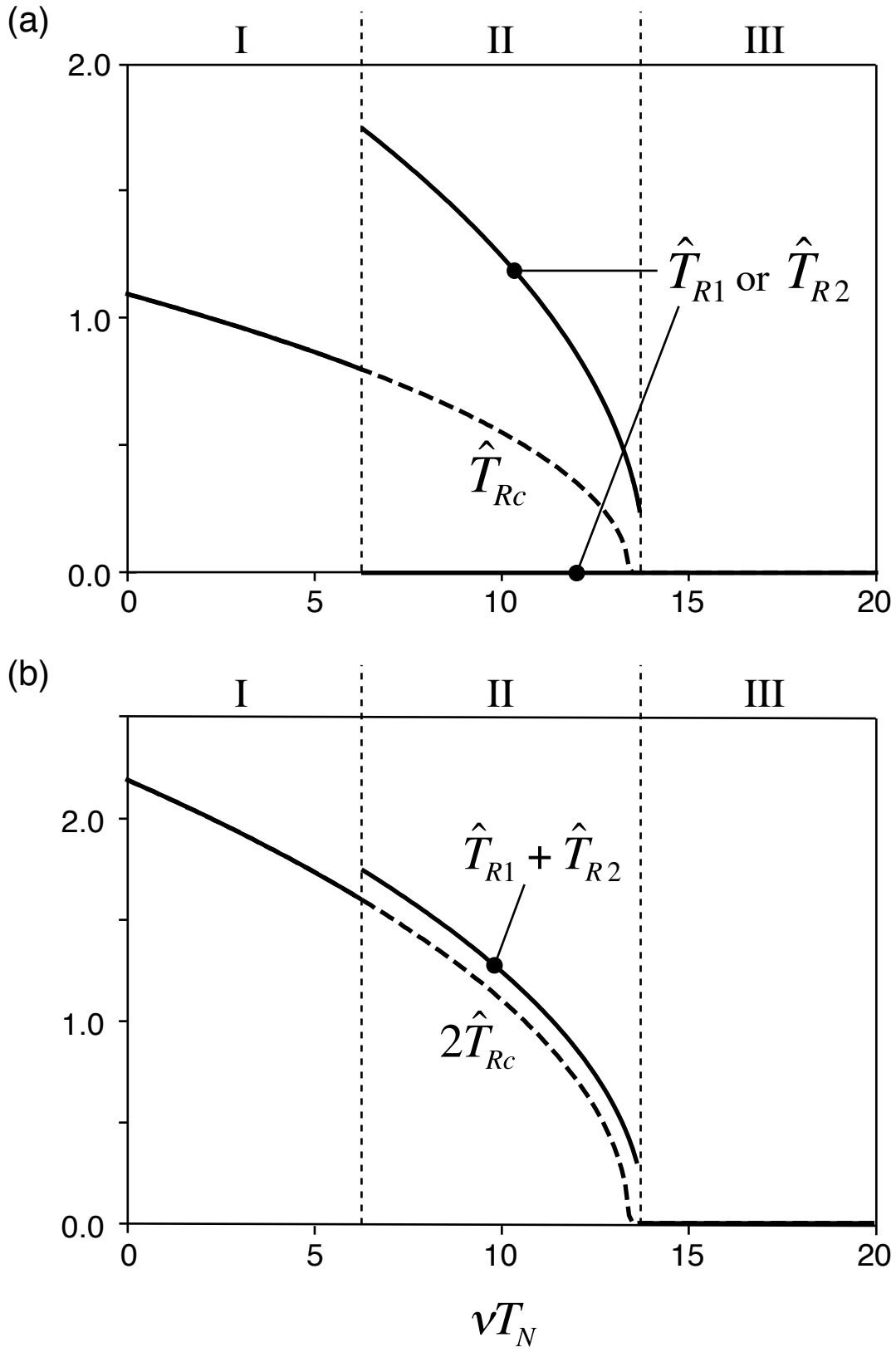

(a)
I
II
III
2.0
1.0
0.0
Segregated
Colocalized
0
5
10
15
20
νT_N
(b)
2.0
1.0
0.0
$\hat{T}_{R2}$
$\hat{T}_{Rc}$
$\hat{T}_{R1}$
0.0
0.05
0.10
0.15
0.20
Abundance of type-1 self-antigen, u_1

Figure 2. 7


(a)
I
II
III
2.0
1.0
0.0
0
5
10
15
20
$\hat{T}_{R1}$ or $\hat{T}_{R2}$
$\hat{T}_{Rc}$
(b)
I
II
III
2.0
1.0
0.0
0
5
10
15
20
$\hat{T}_{R1} + \hat{T}_{R2}$
$2\hat{T}_{Rc}$
νT_N

Chapter 3

T cell anergy as a strategy to reduce the risk of autoimmunity

The study of this chapter was published in

Saeki, K., and Iwasa, Y., *Journal of Theoretical Biology*, **277**, 74-82 in 2011.

Introduction

There is another mechanism to prevent autoimmunity other than regulatory T cells. T cell anergy has been defined as a state of unresponsiveness in T cells associated with nonproliferation and a lack of cytokine production, which can be reversed by interleukin (IL)-2 (Schwartz, 2003). The induction of T cell anergy was first demonstrated by stimulating CD4+ Th1 clones in the absence of a costimulatory signal (Jenkins and Schwartz, 1987; Quill and Schwartz, 1987), but it can occur even in the presence of costimulation (CD28 on T cells with B7 on APCs), depending on the magnitude of the signal from the specific peptide-major histocompatibility complex (MHC). Experimentally, T cell anergy can be induced by weak stimuli from APCs caused by altered peptide ligands (Sloan-Lancaster et al., 1994) or low numbers of agonist ligands on the APC (Korb et al., 1999, Mirshahidi et al., 2001). The induction of T cell anergy by weak stimuli is an important mechanism of self-tolerance that reduces the risk of autoimmunity by suppressing T cell activation (e.g., Grossman and Paul, 2001; Chan et al., 2005; Powell, 2006). However, the general suppression of T cell activation in the periphery is not beneficial to the host because useful T cell activation, which is important for fighting against pathogens (non-self-antigens), is also suppressed.

I argue that T cells that receive weak stimuli from antigens are more likely to be self-reactive than T cells that receive strong stimuli, as follows: Since self-reactive T cells that receive strong stimuli from self-antigens are eliminated in the thymus, T cells that receive strong stimuli in the periphery are likely to be non-self-reactive. As a consequence, when a peripheral T cell receives a weak stimulus, the likelihood that the cell is self-reactive is higher than when it receives a strong stimulus. Therefore, inactivation of T cells that receive weak stimuli may reduce the danger of autoimmunity without harming the ability of the host to defend against pathogens. I examine the hypothesis that T cell anergy is an adaptive response of T cells under uncertainty about whether they are self-reactive or not. In the present model, the risk of autoimmunity and the benefit of maintaining the ability to

attack foreign antigens are explicitly considered, and the optimal T cell response when the cell experiences a weak or a strong stimulus is explored. For some range of parameter values, the optimal response of T cells is to become activated when the stimulus is strong but to be inactivated when the stimulus is weak.

Model

Let suppose the following simplest situation: there are two types of antigens (self-antigen and non-self-antigen), two types of T cells (self-reactive and non-self-reactive), and two levels of the stimulus (weak and strong). Self-reactive T cells recognize non-self-antigens, and non-self-reactive T cells recognize self-antigens, but there is no cross-reactivity. Importantly, each T cell does not know exactly whether it itself is self-reactive or non-self-reactive, but it has to choose a response when it receives a stimulus. I consider the probability that a naive T cell in the periphery receives a weak or a strong stimulus. Because of the negative selection in the thymus, the probability of receiving a weak or a strong stimulus differs between the two types of T cells in the periphery. Let $f(x_1)$ be the probability of receiving a weak stimulus when a naive non-self-reactive T cell interacts with a non-self-antigen. Let $g(x_1)$ be that probability when a naive self-reactive T cell interacts with a self-antigen. In a similar manner, let $f(x_2)$ and $g(x_2)$ be the probabilities for a non-self-reactive and a self-reactive T cell receiving a strong stimulus when interacting with a non-self-antigen and a self-antigen, respectively. Because of normalization, $f(x_1) + f(x_2) = 1$ and $g(x_1) + g(x_2) = 1$.

The origin of the difference in receiving strong/week signals between two types of T cells is explained as follows: Before the negative selection in the thymus, there are many types of T cells, including both self-reactive and non-self-reactive T cells. In addition, some T cells are likely to interact strongly with their specific antigens, and others are likely to interact weakly. I assume that if an immature T cell reactive to non-self-antigens is randomly

chosen, it receives a weak stimulus with probability $f(x_1)$ and a strong stimulus with probability $f(x_2)$ from its specific antigens. Since non-self-reactive T cells are not deleted by the negative selection, these probabilities would be the same in the periphery. In the case of self-reactive T cells, I can also assume that in the thymus the probabilities are the same as those for non-self-reactive T cells, that is, a randomly selected self-reactive T cell receives a weak stimulus with probability $f(x_1)$ and a strong stimulus with probability $f(x_2)$ before the negative selection. However, the negative selection eliminates most self-reactive T cells, and it is especially effective against those that tend to receive strong stimuli from their corresponding antigens. If we focus on those cells that escape the negative selection, the fraction of T cells that receive strong stimuli should be smaller than the original fraction ($f(x_2) > g(x_2)$). In contrast, because of the normalization ($g(x_1) + g(x_2) = 1$), the fraction of T cells that receive weak stimuli should be greater than the original fraction ($f(x_1) < g(x_1)$) (see Fig. 3. 1 for illustration). Hence,

$$\frac{f(x_2)}{g(x_2)} - \frac{f(x_1)}{g(x_1)} > 0 \qquad (3.1)$$

If this quantity is equal to zero, the strength of stimulus is independent of whether the T cell is reactive to non-self-antigens or to self-antigens, and receiving a strong (or a weak) stimulus gives no information as to whether the T cell is reactive to self-antigens. Inequality (3. 1), however, implies that receiving a weak stimulus increases the likelihood of the T cell being reactive to self-antigens, compared to not receiving the signal. In this sense, the strength of the stimulus experienced by a T cell in the periphery carries information concerning the specificity of the T cell.

When a naive T cell receives a stimulus, there are three possible responses: it may become activated, or it may become inactivated (= anergy state), or it may neglect the stimulus (i.e., remain in the naive state). If a naive T cell becomes inactive (T cell anergy), it disregards all subsequent stimuli as long as it is inactive. T cells in the anergic state are functionally inactivated but remain alive for an extended period of time (Schwartz, 2003).

For simplicity of calculation, I first analyze the case in which the anergy state lasts forever, which implies that T cell anergy is equivalent to deletion of the focal cell. Later the other case in which cells in anergy state would return to active again is studied. If a naive T cell keeps the naive state and neglects the stimulus, it will wait for the next encounter with an antigen, or it will die before another encounter.

In general, T cells may make different responses with certain probabilities. In order to represent such a stochastic response, I denote the T cell response strategy by $\sigma_a(x_k)$ and $\sigma_i(x_k)$, where $\sigma_a(x_k)$ is the probability of activation when a naive T cell receives a weak ($k = 1$) or a strong ($k = 2$) stimulus from an antigen, and $\sigma_i(x_k)$ is the probability of inactivation. These probabilities satisfy $0 \le \sigma_a(x_k) + \sigma_i(x_k) \le 1$ ($k = 1, 2$). If a naive T cell is activated, it proliferates and triggers the immune response to eliminate the putative non-self-antigens. Deterministic responses of the T cells can be represented by setting $\sigma_a(x_k)$ and $\sigma_i(x_k)$ equal to either 1 or 0. To consider the optimal response of T cells, the fitness is defined, which consists of a baseline value and the fitness contribution made by all types of T cell. The latter is the sum of the contributions of T cells reactive to non-self-antigens and those reactive to self-antigens:

$$\Phi = \Lambda + \phi_N T_N + \phi_S T_S \qquad (3.2)$$

where Λ is the baseline value of the fitness without T cells, T_N is the abundance of non-self-reactive naive T cells, and T_S is that of self-reactive naive T cells. ϕ_N and ϕ_S are the expected fitness contribution per free naive T cell reactive to a non-self-antigen and a self-antigen respectively, and they are functions of $\sigma_a(x_k)$ and $\sigma_i(x_k)$ ($k = 1, 2$).

The fitness contribution made by one free naive T cell reactive to a non-self-antigen, ϕ_N, can be expanded according to the events occurring in a short time interval of duration Δt. In this time interval, the T cell encounters an APC carrying antigens, or it dies, or it experiences nothing, which occur at probabilities $r\Delta t$, $d\Delta t$, and $(1 - d\Delta t - r\Delta t)$, respectively. Thus, ϕ_N is expanded as,

$$\phi_N = d\Delta t \cdot 0 + r\Delta t \cdot \left[(1-q)\left(\nu \sum_{k=1}^{2} \sigma_a(x_k) f(x_k) + \phi_i \cdot \sum_{k=1}^{2} \sigma_i(x_k) f(x_k) + \right.\right.$$
$$\left.\left. + \phi_N \sum_{k=1}^{2} \left(1 - \sigma_a(x_k) - \sigma_i(x_k)\right) f(x_k) \right) + q\phi_N \right] + (1 - d\Delta t - r\Delta t)\phi_N \qquad (3.3)$$

where q is the frequency of self-antigens. The first term in the right-hand side implies that a free naive T cell does not contribute to the fitness if it dies. The second term indicates the expected fitness when the T cell encounters an APC, and the third term denotes the expected fitness when the T cell experiences nothing in a short time period. In this equation, ν is the payoff when a non-self-reactive naive T cell is activated, ϕ_i in the second term is the payoff when the naive T cell is inactivated, and ϕ_N represents the payoff when the T cell neglects the stimulus. In the first analysis ϕ_i is set to be 0, implying that T cells in anergy state would not return to the naive state. I also assume that a T cell neglects the stimulus when its T cell receptor and the antigen are mismatched, which is expressed in terms of $q\phi_N$ in the large square brackets. From Eq.(3. 3), ϕ_N is calculated as,

$$\phi_N = \frac{\nu(1-q)\sum_{k=1}^{2} \sigma_a(x_k) f(x_k)}{d/r + (1-q)\left(1 - \sum_{k=1}^{2} \left(1 - \sigma_a(x_k) - \sigma_i(x_k)\right) f(x_k)\right)} \qquad (3.4a)$$

where r/d is the average number of encounter between a T cell and an APC. In a similar manner, ϕ_S is expressed as,

$$\phi_S = \frac{-\mu q \sum_{k=1}^{2} \sigma_a(x_k) g(x_k)}{d/r + q\left(1 - \sum_{k=1}^{2} \left(1 - \sigma_a(x_k) - \sigma_i(x_k)\right) g(x_k)\right)} \qquad (3.4b)$$

where $-\mu$ is the payoff when a self-reactive naive T cell is activated. Thus, from Eqs.(3. 3), (3. 4a), and (3. 4b), fitness depends on the response strategy, $\sigma_a(x_k)$ and $\sigma_i(x_k)$ $(k = 1, 2)$.

Optimal reactions in response to stimulation strength

For simplicity of analysis I first consider strategies in which the reaction of the T cell to a stimulus of a given strength is deterministically defined, that is, $\sigma_a(x_k)$ and $\sigma_i(x_k)$ are equal to either 1 or 0, which I refer to as "pure strategies". First, suppose the following three strategies in which T cells are always activated when they receive a strong stimulus ($\sigma_a(x_2)=1$, $\sigma_i(x_2)=0$). These three strategies differ in the reaction to a weak stimulus:

(A) Activation: $\sigma_a(x_1)=1,\ \sigma_i(x_1)=0.$

(B) Inactivation: $\sigma_a(x_1)=0,\ \sigma_i(x_1)=1.$

(C) Neglect the stimulus: $\sigma_a(x_1)=0,\ \sigma_i(x_1)=0.$

In addition to these three, let consider the following two, in which cells are never activated:

(D) No response to either stimulus: $\sigma_a(x_k)=\sigma_i(x_k)=0$ $(k=1,2)$.

(E) Neglect strong stimuli and become inactivated in response to weak stimuli:

$$\sigma_a(x_1)=0,\ \sigma_i(x_1)=1,\ \sigma_a(x_1)=0,\ \sigma_i(x_2)=0.$$

The fitness functions for these five pure strategies are,

(A) Always become activated:

$$\Phi_A=\Lambda+\frac{T_N\nu(1-q)}{d/r+1-q}-\frac{T_S\mu q}{d/r+q} \tag{3.5a}$$

(B) Become activated in response to a strong stimulus and inactivated in response to a weak one:

$$\Phi_B=\Lambda+\frac{T_N\nu(1-q)f(x_2)}{d/r+1-q}-\frac{T_S\mu q g(x_2)}{d/r+q} \tag{3.5b}$$

(C) Become activated in response to a strong stimulus and neglect a weak one:

$$\Phi_C=\Lambda+\frac{T_N\nu(1-q)f(x_2)}{d/r+(1-q)f(x_2)}-\frac{T_S\mu q g(x_2)}{d/r+q g(x_2)} \tag{3.5c}$$

(D) No response to either a strong or a weak stimulus:

$$\Phi_D=\Lambda \tag{3.5d}$$

(E) Neglect a strong stimulus and become inactivated in response to a weak one:

$$\Phi_E=\Lambda \tag{3.5e}$$

By comparing the fitness among these cases, four distinct parameter regions in which each

of the different strategies is the best are obtained in the q-$T_S\mu/T_N\nu$ plane (Fig. 3. 2). The horizontal axis is the fraction of self-antigens q, and the vertical axis is $T_S\mu/T_N\nu$, that is, the risk of autoimmunity relative to the advantage gained by eliminating non-self-antigens (derivation of the boundaries in Fig. 3. 2 is explained in Appendix A).

Strength-dependent inactivation occurs when strategy (B) is better than any of the others, which occurs when the following three conditions are satisfied:

$$\frac{T_S\mu}{T_N\nu} > \frac{d/r+q}{d/r+1-q}\frac{(1-q)f(x_1)}{qg(x_1)} \tag{3. 6a}$$

$$\frac{T_S\mu}{T_N\nu} > \frac{d/r+q}{d/r+1-q}\frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)^2 f(x_1)f(x_2)}{q^2 g(x_1)g(x_2)} \tag{3. 6b}$$

$$\frac{T_S\mu}{T_N\nu} < \frac{d/r+q}{d/r+1-q}\frac{(1-q)f(x_2)}{qg(x_2)} \tag{3. 6c}$$

These conditions establish the boundaries between region B and the other regions in Fig.3. 2. If condition (3. 6a) is reversed but (3. 6b) and (3. 6c) hold, then the optimal strategy is A, in which T cells are activated both by a strong and by a weak stimulus. If condition (3. 6b) is reversed instead, and the other two conditions hold, then the optimal strategy is C, in which cells are activated by a strong stimulus but neglect a weak stimulus. If condition (3. 6c) is reversed but the other two hold, the optimal strategy becomes either D or E. In both these strategies, T cells neglect a strong stimulus and the fitness outcome is the same.

In general, the most aggressive strategy (strategy A) is optimal when $T_S\mu/T_N\nu$ is very small, and a more defensive strategy (strategy B or C) becomes optimal as self-reactive T cells become more frequent or as the disadvantage of self-reactive T cells being activated becomes larger. If $T_S\mu/T_N\nu$ becomes very large, then the optimal pure strategy becomes either D or E, which means that having T cells is no longer beneficial. There are two characteristic points, $\hat{q}_1$ and $\hat{q}_2$, which determine whether strategy B or C can be the optimal strategy. When $0 < q \le \hat{q}_1$, strategy A, C, or D can be optimal. When $\hat{q}_1 \le q < \hat{q}_2$, all five strategies can be optimal. When $\hat{q}_2 \le q < 1$, strategy A, B, or D can be optimal (the derivations of $\hat{q}_1$ and $\hat{q}_2$ are also in Appendix A).

Parameter dependence

The condition in which region B, implying that inactivation is the optimal response, is wide in the parameter space. The size of region B is calculated by the following integral

$$\frac{f(x_2)}{g(x_2)}\int_{\hat{q}_1}^{\hat{q}_2}\left[\frac{d/r+q}{d/r+1-q}\frac{(1-q)}{q}\left(1-\frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)f(x_1)}{qg(x_1)}\right)\right]dq$$
$$+\int_{\hat{q}_2}^{1}\left[\frac{(1-q)}{q}\left(\frac{f(x_2)}{g(x_2)}-\frac{f(x_1)}{g(x_1)}\right)\right]dq$$

where $\hat{q}_2 = f(x_2)/\left(f(x_2)+g(x_2)\right)$ and $\hat{q}_1$ is the value of q at which the right hand side of conditions (3. 6b) and (3. 6c) become equal to each other (see also Appendix A). The numerical integration shows that the region of anergy becomes wider as r/d increases (Fig. 3. 3). When r/d is very large, $\hat{q}_1$ becomes nearly 0, and the integral is approximately equal to the following

$$\begin{bmatrix}\text{Area in wich anergy}\\ \text{is the optimal.}\end{bmatrix} \approx \left(\frac{f(x_2)}{g(x_2)}-\frac{f(x_1)}{g(x_1)}\right)\left(2\hat{q}_2-1-\ln\hat{q}_2\right) \qquad (3.7)$$

Because the second factor on the right hand side is positive (since $1/2 < \hat{q}_2 < 1$), Eq.(3. 7) indicates that T cell anergy can be the optimal response only when condition (3. 1) holds. The quantity r/d is the expected number of encounters with antigens of a free naive T cell in its lifetime. In an animal body, it is likely to be large because naive T cells will experience many encounters with many different antigens.

I summarize the conditions that are likely to result in T cell anergy in the case of deterministic responses as follows: First, T cell anergy is more likely to be the optimal strategy when $T_S\mu/T_N\nu$ is of an intermediate magnitude. This condition implies that the risk of autoimmunity relative to the advantage gained by eliminating non-self-antigens is of intermediate magnitude. If the consequence of responding to self-antigens are too severe or self-reactive T cells are too abundant, no response becomes optimal. In contrast, if the consequence of responding to self-antigens is less severe or self-reactive T cells are rare, the

optimal strategy is to always become activated in response to stimuli of any strength. Second, T cell anergy is more likely to occur when *r*/*d* is large, which is quite likely to be the case in a body. Third, for T cell anergy to occur, condition (3. 1) must hold, which is also quite plausible. Finally, q must be larger than a certain threshold ($\hat{q}_1$).

Mixed strategies

The reaction of a T cell to a given stimulus (either weak or strong) was assumed to make one of three responses (activate, inactivate, or neglect) in a deterministic manner. Although these "pure strategies" are intuitively appealing as candidates for optimal behavior, T cells respond stochastically to stimuli (Karttunen and Shastri, 1991). In this section, I consider stochastic responses or "mixed strategies", in which different reactions are adopted with intermediate probabilities. The mixed strategies are expressed in terms of intermediate values of $\sigma_a(x_k)$ and $\sigma_i(x_k)$ ($0 \le \sigma_a(x_k) \le 1$, $0 \le \sigma_i(x_k) \le 1$, and $0 \le \sigma_a(x_k) + \sigma_i(x_k) \le 1$, for $k = 1, 2$). To examine the possibility that a mixed strategy is better than any of the pure strategies, I conducted an exhaustive numerical search for optimal values of $\sigma_a(x_k)$ and $\sigma_i(x_k)$ ($k = 1$, 2) that would maximize the fitness, given as,

$$\Phi = \Lambda + \frac{T_N \nu (1-q) \sum_{k=1}^{2} \sigma_a(x_k) f(x_k)}{d/r + (1-q)\left(1 - \sum_{k=1}^{2} \left(1 - \sigma_a(x_k) - \sigma_i(x_k)\right) f(x_k)\right)} - \frac{T_S \mu q \sum_{k=1}^{2} \sigma_a(x_k) g(x_k)}{d/r + q\left(1 - \sum_{k=1}^{2} \left(1 - \sigma_a(x_k) - \sigma_i(x_k)\right) g(x_k)\right)} \qquad (3.8)$$

The pure strategies are special cases of mixed strategies in which $\sigma_a(x_k)$ and $\sigma_i(x_k)$ are either 0 or 1. In order to search for the optimal T cell response when T cells can follow mixed strategies, fitness Eq.(3. 8) is calculated for different sets of the four variables, $\sigma_a(x_k)$ and $\sigma_i(x_k)$ ($k = 1, 2$), made by changing their values from 0 to 1 in 0.001

increments. Then the optimal values of $\sigma_a(x_k)$ and $\sigma_i(x_k)$ ($k = 1, 2$) are obtgained on the q-$T_S\mu/T_N\nu$ plane.

The results clearly show that a mixed strategy is better than any of the pure strategies for a wide range of parameters (Fig. 3. 4). There are some areas in which the optimal strategy is a mixture of two of the five pure strategies. A mixed strategy can be optimal between two different regions in which different pure strategies are the best. For example, a T cell following the mixed strategy B + E is activated with an intermediate probability when it receives a strong stimulus and inactivated when it receives a weak stimulus, and a T cell following the mixed strategy B + C is activated when it receives a strong signal and inactivates with an intermediate probability when it receives a weak stimulus. In Fig. 3. 4, the contours in the B + C region denote the optimal value of $\sigma_i(x_1)$, and those in the B + E region denote the optimal value of $\sigma_a(x_2)$, and the numbers accompanying the contours indicate the fraction of B strategy in the optimal mixture. T cell anergy will be the optimal response in the areas designated as B + E, B, and B + C. The size of the region in which the "pure B strategy" is optimal in this scenario (mixed strategies are allowed) is much smaller than it is in the previous scenario (where reactions are restricted to pure strategies). However, the total size of the region in which T cell anergy occurs with a positive probability (i.e., B + E, B, and B + C combined) seems to be larger. Interestingly, T cell anergy is not observed in the optimal strategy if q is below a threshold value, which can be seen as a vertical line in Fig. 3. 4 forming a boundary between different regions. As a result, to achieve T cell anergy, it is more important for q to be large when mixed strategies are allowed than when only pure strategies are allowed.

When T cells in anergy state return to the naive state

In the anergic state, T cells are functionally inactivated, but remain alive for an extended period of time. I next consider the situation in which once inactivated T cells may return to

the naive state at rate b. I also assume that cells in anergy may have a death rate, denoted by d', smaller than naive T cells (i.e., $d' < d$). Note the expected fitness contribution of a non-self-reactive T cell in anergy, ϕ_i^N, was set to be 0 in the previous section. Now a formula for ϕ_i^N is calculated by the expansion of events occurring in a shot time period of length Δt,

$$\phi_i^N = d'\Delta t \cdot 0 + b\Delta t \cdot \phi_N + (1 - d'\Delta t - b\Delta t)\phi_i^N \qquad (3.9)$$

Then $\phi_i^N = b\phi_N / (d'+b)$ is obtained, and the fitness contribution of one free naive T cell reactive to a non-self-antigen, ϕ_N, is rewritten as

$$\phi_N = \frac{\nu(1-q)\sum_{k=1}^{2}\sigma_a(x_k)f(x_k)}{d/r + (1-q)\left(1 - \frac{b}{d'+b}\sum_{k=1}^{2}\sigma_i(x_k)f(x_k) - \sum_{k=1}^{2}\left(1-\sigma_a(x_k)-\sigma_i(x_k)\right)f(x_k)\right)} \qquad (3.10a)$$

which has a term in the denominator that was absent in Eq.(3. 3a). In a similar manner, the fitness contribution of a self-reactive T cell in anergic state is $\phi_i^S = b\phi_S / (d'+b)$, and the formula for ϕ_S is rewritten as

$$\phi_S = \frac{-\mu q\sum_{k=1}^{2}\sigma_a(x_k)g(x_k)}{d/r + q\left(1 - \frac{b}{d'+b}\sum_{k=1}^{2}\sigma_i(x_k)g(x_k) - \sum_{k=1}^{2}\left(1-\sigma_a(x_k)-\sigma_i(x_k)\right)g(x_k)\right)} \qquad (3.10b)$$

When each T cell can respond deterministic manner (only pure strategies are allowed), I can calculated the condition in which inducing anergy is the optimal response as in Appendix B.

The size of region in which inducing anergy is the optimal (region B) is shown with the deterministic model (Fig. 3. 5). When d'/b is large ($d'/b >> 1$), the size of region B increases with r/d, as in the last section. In contrast when d'/b is small ($d'/b << 1$), the size of region B does not increase monotonically with r/d. When the stochastic responses of T cells allowed for weak or strong stimuli, the optimal responses can be calculated numerically and the result also depends on the ratio d'/b. The region in which inducing inactivation looks similar to Fig. 3. 4a with $d'/b = 1$ (Fig. 3. 6a). With lower value of d'/b, however, the region appears to be

smaller, and especially the upper right region (both $T_S\mu/T_N\nu$ and q are large) disappears (Fig. 3. 6b, $d'/b = 0.01$). The result shows that the advantage of inducing anergy state critically depends on the ratio of the death rate in the anergy state d' to the rate of returning to naive state b. If d'/b is large, most of anergic T cells will die before returning to the naive state, and the situation is similar to the last section. In contrast, if d'/b is low, many T cells in anergy state will back to naive state. Then inducing T cell anergy is not very effective as a measure to reduce self-reactive T cells selectively. The advantage of inducing anergy is limited especially when the frequency of self-reactive T cells is high (high $T_S\mu/T_N\nu$) or that of self-antigens are high (high q).

Discussion

I examined the possibility that T cell anergy might be an adaptive reaction to reduce the risk of autoimmunity under the situation that each T cell cannot know for sure whether it is reactive to foreign antigens or to self-antigens. To examine the adaptive significance of T cell anergy, I adopted the formalism that each T cell chooses its response depending on the strength of stimuli and maintains the ability to attack foreign antigens while reducing the risk of autoimmunity. The response of a T cell when it encounters an antigen is to become activated or to become inactivated (i.e. anergy), or to neglect the stimulus, and in general the response may depend on the strength of the stimulus that the T cell experiences. The optimal T cell responses to weak and strong stimuli are determined by considering the benefit of activating non-self-reactive T cells and the harm of activating self-reactive T cells.

The analysis demonstrated that T cell anergy is likely to be optimal when the following conditions are satisfied: [1] the frequency of self-antigens is high (large q), [2] a naive T cell meets antigens many times in its lifetime (large r/d), [3] the product of the harm caused by autoimmunity and the number of self-reactive T cells relative to the product of the benefit to eliminate pathogens and the number of non-self-reactive T cells has an intermediate

magnitude (intermediate $T_S\mu/T_N\nu$), and [4] the condition (3. 1) holds. Among these, condition [1] is plausible in a body because a large fraction of antigens are self-antigens in tissues without any infections caused by pathogens. Condition [2] is also quite plausible because the half-life of naive phenotype T cells has been measured using ^2H_2O incorporation to be approximately 2 years in adult humans (Neese et al., 2002). During this time, naive T cells must experience many encounters with many different APCs, which suggests that r/d is large in a body.

Condition [3] is also plausible but it is more difficult to confirm. Because of the efficiency of the negative selection in the thymus, the number of self-reactive T cells is likely to be much smaller than the number of non-self-reactive T cells ($T_S << T_N$). On the other hand, the harm to the host caused by autoimmunity is much greater than the benefit of having a T cell that is useful for fighting foreign antigens ($\mu > \nu$). In this situation, $T_S\mu/T_N\nu$ can have an intermediate magnitude. If the quantity is very large, then the possibility of activating self-reactive T cells by mistake becomes large (large T_S) or the damage caused by autoimmunity is extremely severe (very large μ). In this situation, no activation is optimal and having T cells is not beneficial. In contrast, if $T_S\mu/T_N\nu$ is very small, the optimal response for a T cell is to become activated irrespective of the strength of the stimulus because the risk of autoimmunity becomes too low to be worth caring about. Finally, it is plausible to assume that the negative selection in the thymus removes high-affinity self-reactive T cells more effectively than low-affinity self-reactive T cells. The hypothesis leads to the condition (3. 1), which suggests that self-reactive T cells are more likely to receive weak stimuli than non-self-reactive T cells compared to non-self-reactive T cells. This might be tested experimentally by the use of transgenic mice and the analysis of T cell repertoire (e.g., Jordan et al., 2001; Bautista et al., 2009; Li et al., 2010; Valkenburg et al., 2010). For example, in transgenic mice which have genome inserted genes for a particular peptide and its corresponding T cell receptor (TCR), T cells bearing the transgenic TCR will be self-reactive. In contrast, in mice which are inserted a TCR gene only, T cells bearing the

transgenic TCR will be non-self-reactive. We may be able to compare the affinity for the corresponding antigen between self-reactive T cells and non-self-reactive T cells.

I also calculated the optimal T cell responses when T cells are allowed to adopt stochastic reactions to a given stimulus, that is, T cells choose among multiple responses according to a probability distribution. If such mixed strategies are allowed, then the optimal response of T cells can be a mixed strategy (instead of a pure strategy) for a wide range of parameter values. The parameter range in which T cells show anergy with a positive probability is wider than the range when the reaction is deterministically determined.

Since T cells once entering in anergy state may go back to the naive state, I also studied the situation that anergic T cells return to naive at a constant rate of b. Returning from inactive to active state would contribute to the defense against pathogens if the T cell is not self-reactive, although it can be a self-reactive T cell causing autoimmunity later. The analysis of the model shows that when anergic T cells are more likely to return to naive, inducing anergy is less likely to be beneficial. There are two possibilities for the advantages of keeping suspicious T cells alive and allow them to become activated again later instead of the immediate elimination. First, ratio d'/b is really large. This possibility cannot be excluded at this moment because the reliable quantitative estimate on the value of the ratio d'/b have not been obtained, although there are measurements on the length of anergy state (Rocha et al. 1995; Pape et al. 1998; Ryan and Evavold 1998). Second, d'/b may not be very large, but the other parameters, $T_S\mu/T_N\nu$ and q, are in the region in which anergy is the optimal. Finally, considering environmental fluctuations, keeping suspected self-reactive T cells alive to be better than immediate killing. Whether to activate or to remove T cells with some likelihood of being self-reactive should depend on the relative magnitude of the risk of autoimmunity and the need of preventing harmful invading pathogens. When the immune system detects signals indicating the invasion of pathogens to a body, the need of fighting external antigens becomes enhanced and can exceed the estimated risk of autoimmunity. This conjecture may be supported by the observation that T cells in anergy become naive state again in the

presence of IL2, which is a chemical signal indicating the invasion of pathogens (Beverly et al. 1992).

The analysis is limited to the simplest case, but there are many different ways to extend the analysis. Although I focus on the very simple case of stimuli of two different levels (weak or strong), the probability of T cell activation differs depending on more than two levels of stimuli (Karttunen and Shastri, 1991). Thus the analysis of the optimal responses should also include this aspect. I also assumed that the T cells respond to the strength of the current stimulus only. However, in general T cells may memorize the stimuli experienced in past encounters. If so, decision making by T cells may depend on their history as well as on the current stimulus. For example, Grossman and Paul (2001) proposed the existence of a "tunable activation threshold (TAT)", according to which T cells tune up their activation thresholds in response to recurrent stimuli (see also Carneiro et al., 2005). Several experiments have indicated that T cell sensitivity might change dynamically (Smith et al., 2001; Tanchot et al., 2001; Wong et al., 2001; Marquez et al., 2005). Defining fitness and examining the benefit and disadvantrage will help us to understand the significance of these phenomena.

Appendix A: Comparison of pure strategies

The fitness of each five pure strategies are given by Eq.(3. 5), and their magnitude relations are as follows:

$\Phi_A > \Phi_B$ when

$$\frac{\mu T_S}{\nu T_N} < \frac{d/r+q}{d/r+1-q}\frac{(1-q)f(x_1)}{qg(x_1)} \tag{3. 11}$$

$\Phi_B > \Phi_C$ when

$$\frac{\mu T_S}{\nu T_N} > \frac{d/r+q}{d/r+1-q}\frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)^2 f(x_1)f(x_2)}{q^2 g(x_1)g(x_2)} \tag{3. 12}$$

$\Phi_C > \Phi_A$ when

$$\frac{\mu T_S}{\nu T_N} > \frac{d/r+q}{d/r+1-q}\frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)f(x_1)}{qg(x_1)} \tag{3. 13}$$

$\Phi_A > \Phi_D = \Phi_E$ when

$$\frac{\mu T_S}{\nu T_N} < \frac{d/r+q}{d/r+1-q}\frac{1-q}{q} \tag{3. 14}$$

$\Phi_B > \Phi_D = \Phi_E$ when

$$\frac{\mu T_S}{\nu T_N} < \frac{d/r+q}{d/r+1-q}\frac{(1-q)f(x_2)}{qg(x_2)} \tag{3. 15}$$

$\Phi_C > \Phi_D = \Phi_E$ when

$$\frac{\mu T_S}{\nu T_N} < \frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)f(x_2)}{qg(x_2)} \tag{3. 16}$$

By comparing these conditions, the optimal strategy is obtained in the q-$T_S\mu/T_N\nu$ plane. The boundary between two neighboring regions is obtained by setting the corresponding inequality to equation.

For $0 < q < 1$, there are two points at which three regions meet:

$$\hat{q}_1 = \frac{f(x_2)-g(x_2)+(f(x_1)+g(x_1))d/r}{2(f(x_2)-g(x_2))} - \frac{\sqrt{\left(f(x_2)-g(x_2)+(f(x_1)+g(x_1))d/r\right)^2 - 4f(x_1)(f(x_2)-g(x_2))d/r}}{2(f(x_2)-g(x_2))} \tag{3. 17}$$

at which the right hand side of (3. 12), (3. 15), and (3. 16) become equal, and

$$\hat{q}_2 = \frac{f(x_2)}{f(x_2)+g(x_2)} \tag{3. 18}$$

at which the right hand side of (3. 11), (3. 12), and (3. 13) become equal. According to the conditions (3. 11)–(3. 16), "Always Activate" or "Activate and Neglect" or "No activation" can be optimal when $0 \le q \le \hat{q}_1$. When $\hat{q}_1 \le q < \hat{q}_2$, all five strategies can be optimal. When $\hat{q}_2 < q \le 1$, "Always Activate" or "Activate and Inactivate" or "No activation" can be optimal.

Appendix B

Consider the situation in which once inactivated T cells may return to the naive state. From Eq.(3. 9), the fitness of strategy B, inducing T cell anergy for a weak stimulus, is

$$\Phi_B = \Lambda + \frac{T_N \nu (1-q) f(x_2)}{d/r + (1-q)\left(1 - \frac{bf(x_1)}{d'+b}\right)} - \frac{T_S \mu q g(x_2)}{d/r + q\left(1 - \frac{bg(x_1)}{d'+b}\right)} \qquad (3.19)$$

The fitnesses of the other four pure strategies are the same as shown by Eq.(3. 5). The q-$T_S\mu/T_N\nu$ plane is divided into four different regions in which the best strategy is A (Φ_A is the largest), B (Φ_B is the largest), C (Φ_C is the largest), or D or E ($\Phi_D = \Phi_E$ is the largest). There are several characteristic values of q, denoted by $\hat{q}_1$, $\hat{q}_3$, and $\hat{q}_4$, at which three regions meet (I do not use symbol $\hat{q}_2$ to avoid possible confusion with the one in Appendix A). When $0 < q \le \hat{q}_1$, strategy A, C, or D can be optimal. When $\hat{q}_1 < q \le \hat{q}_3$, all five strategies can be optimal. When $\hat{q}_3 < q \le \hat{q}_4$, strategy A, B, or D can be optimal. Finally, when $\hat{q}_4 < q < 1$, strategy A, or D can be optimal. The $\hat{q}_1$ and $\hat{q}_3$ always exist between 0 and 1, but $\hat{q}_4$ may or may not exist.

The first characteristic point $\hat{q}_1$ is the same in the case with $b = 0$ (shown in Appendix A). The second point $\hat{q}_3$, which is different from $\hat{q}_2$ in Appendix A, is derived by solving $\Phi_A = \Phi_B = \Phi_C$ with respect to q. The equation that $\hat{q}_3$ should satisfy is $H(q) = 0$ where

$$H(q) = \left\{2f(x_2)g(x_2) + \frac{d'}{b}(f(x_2)+g(x_2))\right\}q^3$$
$$+\left\{\frac{d}{r}(f(x_2)-g(x_2)) - 3f(x_2)g(x_2) - \frac{d'}{b}(2f(x_2)+g(x_2))\right\}q^2$$
$$+\left\{f(x_2)g(x_2) - \frac{d}{r}(f(x_2)+g(x_2))\left(\frac{d}{r}\left(\frac{d'}{b}+1\right)-\frac{d'}{b}\right) + f(x_2)\left(\frac{d'}{b}-2\frac{d}{r}\right)\right\}q$$
$$+\frac{d}{r}\left(\frac{d'}{b}+1\right)\left(\frac{d}{r}+1\right)f(x_2)$$

By using $f(x_2) > g(x_2)$, $H(0) > 0$ and $H(1) < 0$ are apparent, and then $0 < \hat{q}_3 < 1$ hold.

The third characteristic value $\hat{q}_4$ is derived by solving equations which satisfy $\Phi_A = \Phi_B = \Phi_D = \Phi_E$. The equation that $\hat{q}_4$ should satisfy is $J(q) = 0$ where

$$J(q) = \frac{d'}{b}(f(x_2)-g(x_2))q^2$$
$$-\left\{\frac{d'}{b}(f(x_2)-g(x_2)) - \frac{d}{r}(f(x_1)g(x_2)+f(x_2)g(x_1))\right\}q.$$
$$-\frac{d}{r}\left\{f(x_2)g(x_1) + \left(\frac{d}{r}\left(\frac{d'}{b}+1\right)+\frac{d'}{b}\right)(f(x_2)-g(x_2))\right\}$$

Since $J(0) < 0$, the condition for $0 < q_4 < 1$ is

$$\frac{d}{r} < \frac{f(x_1)g(x_2)}{f(x_2)-g(x_2)} \tag{3.20}$$
$$\frac{d'}{b} < \frac{f(x_1)g(x_2)-(f(x_2)-g(x_2))d/r}{(f(x_2)-g(x_2))(1+d/r)} \tag{3.21}$$

The size of region B when conditions (3. 20) and (3. 21) hold is calculated by the following integral:

$$\frac{f(x_2)}{g(x_2)}\int_{\hat{q}_1}^{\hat{q}_3}\left[X\frac{(1-q)}{q}\left(1-\frac{d/r+qg(x_2)}{d/r+(1-q)f(x_2)}\frac{(1-q)f(x_1)}{qg(x_1)}\right)\right]dq$$
$$+\int_{\hat{q}_3}^{\hat{q}_4}\left[X\frac{(1-q)}{q}\left(\frac{f(x_2)}{g(x_2)}-\frac{d/r+q}{d/r+(1-q)}\frac{f(x_1)}{g(x_1)}\frac{(d'+b)d/r+(1-q)d'}{(d'+b)d/r+qd'}\right)\right]dq$$

where X is

$$X = \frac{(d'+b)d/r+q(d'+bg(x_2))}{(d'+b)d/r+(1-q)(d'+bf(x_2))}$$

When either (3. 20) or (3. 21) is not satisfied, the range of integration of the second term is from $\hat{q}_3$ to 1.

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Figure Legends

Figure 3. 1 The probabilities of receiving a weak or a strong stimulus differ between self-reactive T cells and non-self-reactive T cells because negative selection in the thymus preferentially removes self-reactive T cells that tend to receive strong stimuli from their corresponding antigen.

Figure 3. 2 Optimal pure strategies on the q-$T_S\mu/T_N\nu$ plane. The horizontal axis is the frequency of self-antigens q, and the vertical axis is the risk of autoimmunity relative to the benefit of non-self-reactive T cells ($T_S\mu/T_N\nu$). The four labeled regions are as follows: A, the cell always becomes activated; B, inducing inactivation by a weak stimulus; C, the cell is activated by a strong stimulus and neglects a weak one; and D, E (which have the same fitness). (b) Enlargement of the area where $0 < q < 0.02$. (c) Enlargement of the area where $0.8 < q < 1$. $\hat{q}_1$ and $\hat{q}_2$ were calculated by the using the following parameter values: $r/d = 100, f(x_1) = f(x_2) = 0.5, g(x_1) = 0.95, g(x_2) = 0.05$ (see Appendix A).

Figure 3. 3 The area of region B in which T cell anergy is optimal in relation to the expected number of encounters with antigens of a free naive T cell in its lifetime, r/d. Three lines are obtained by using the following parameter sets: (I) $f(x_1) = f(x_2) = 0.5, g(x_1) = 0.95, g(x_2) = 0.05$. (II) $f(x_1) = f(x_2) = 0.5, g(x_1) = 0.9, g(x_2) = 0.1$. (III) $f(x_1) = 0.7, f(x_2) = 0.3, g(x_1) = 0.95, g(x_2) = 0.05$.

Figure 3. 4 Optimal mixed strategies on the q-$T_S\mu/T_N\nu$ plane. The horizontal and the vertical axes are the same as in Fig. 3. 2. (a) The regions are indicated by letters or combinations of two letters (see text). T cell anergy is included in regions B + E, B, and B + C. The thick lines are the boundaries between regions. In regions B + E and B + C, the thin lines are contours showing the fractions of the two component strategies, with the numbers indicating the fraction of strategy B in the optimal mixture. (b) Enlargement of the area near

the horizontal axis. The optimal responses shown in this figure are A, A + C, B + C, and C + D. As in (a), the thick lines are the boundaries between regions, and the thin lines are contours. Other parameters are: $r/d = 100, f(x_1) = f(x_2) = 0.5, g(x_1) = 0.95, g(x_2) = 0.05$.

Figure 3. 5 Contour map for the size of region B in which T cell anergy is optimal when some cells in anergy state go back to naive sate later ($d'/b > 0$). Horizontal axis is the expected number of encounters with antigens of a free naive T cell, r/d, and the vertical axis is the ratio of the death rate in the anergy state to the rate of returning to naive state, d'/b. Other parameters are: $f(x_1) = f(x_2) = 0.5, g(x_1) = 0.95, g(x_2) = 0.05$.

Figure 3. 6 Optimal mixed strategies on the q-$T_S\mu/T_N\nu$ plane when $d'/b > 0$. The horizontal and the vertical axes are the same as in Fig. 3. 4. Each region is indicated by letters or combinations of two letters, and thick lines indicate the boundaries between regions. In regions B + E and B + C, the thin lines are contours for the fractions of the two component strategies, with the numbers indicating the fraction of strategy B in the optimal mixture. (a) $d'/b = 1$, (b) $d'/b = 0.01$. Other parameters are: $r/d = 100, f(x_1) = f(x_2) = 0.5, g(x_1) = 0.95, g(x_2) = 0.05$.

Figure 3. 1

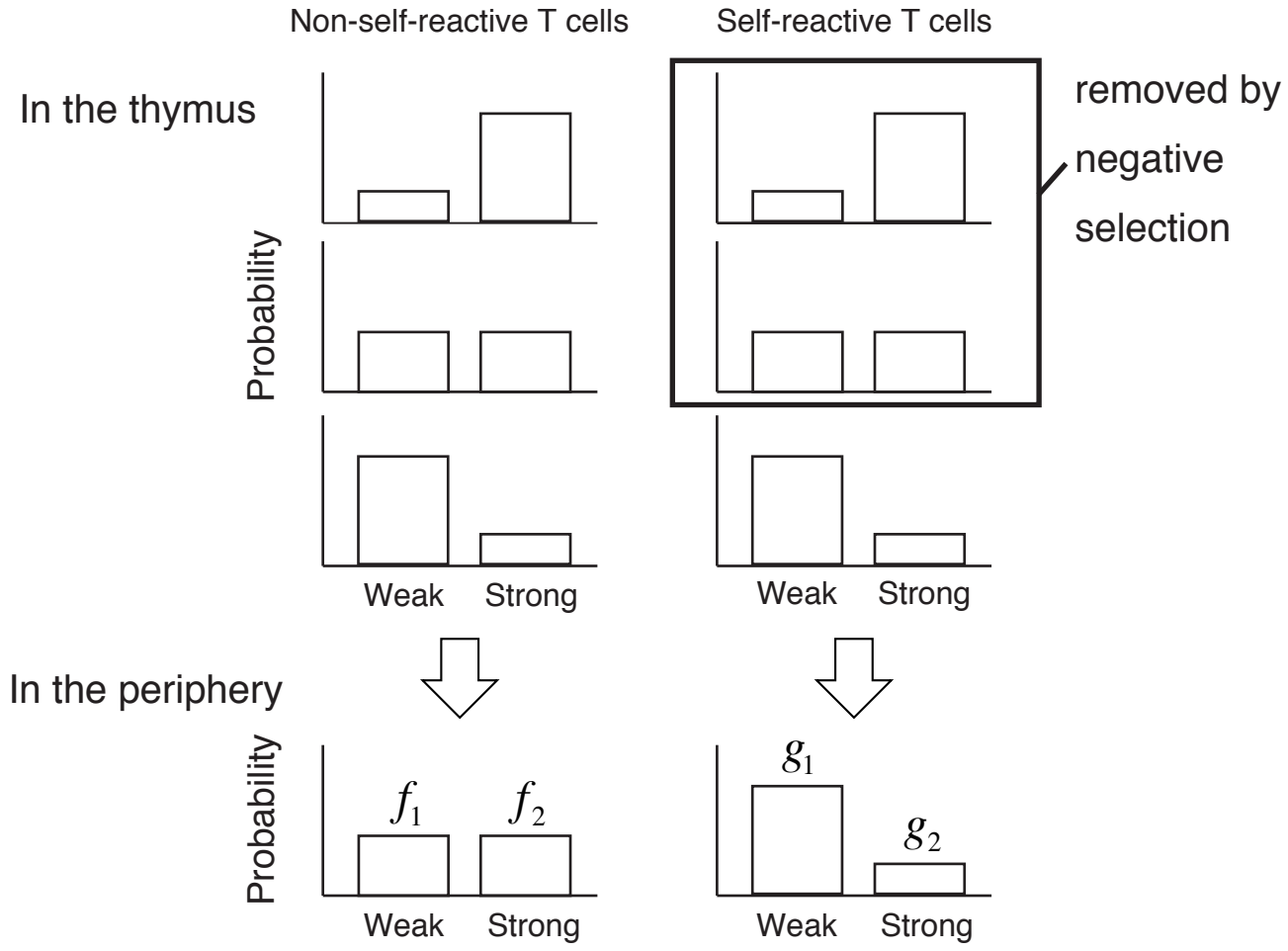

Non-self-reactive T cells
Self-reactive T cells
In the thymus
removed by
negative
selection
Probability
Weak
Strong
Weak
Strong
In the periphery
Probability
f_1
f_2
g_1
g_2
Weak
Strong
Weak
Strong

Figure 3. 2

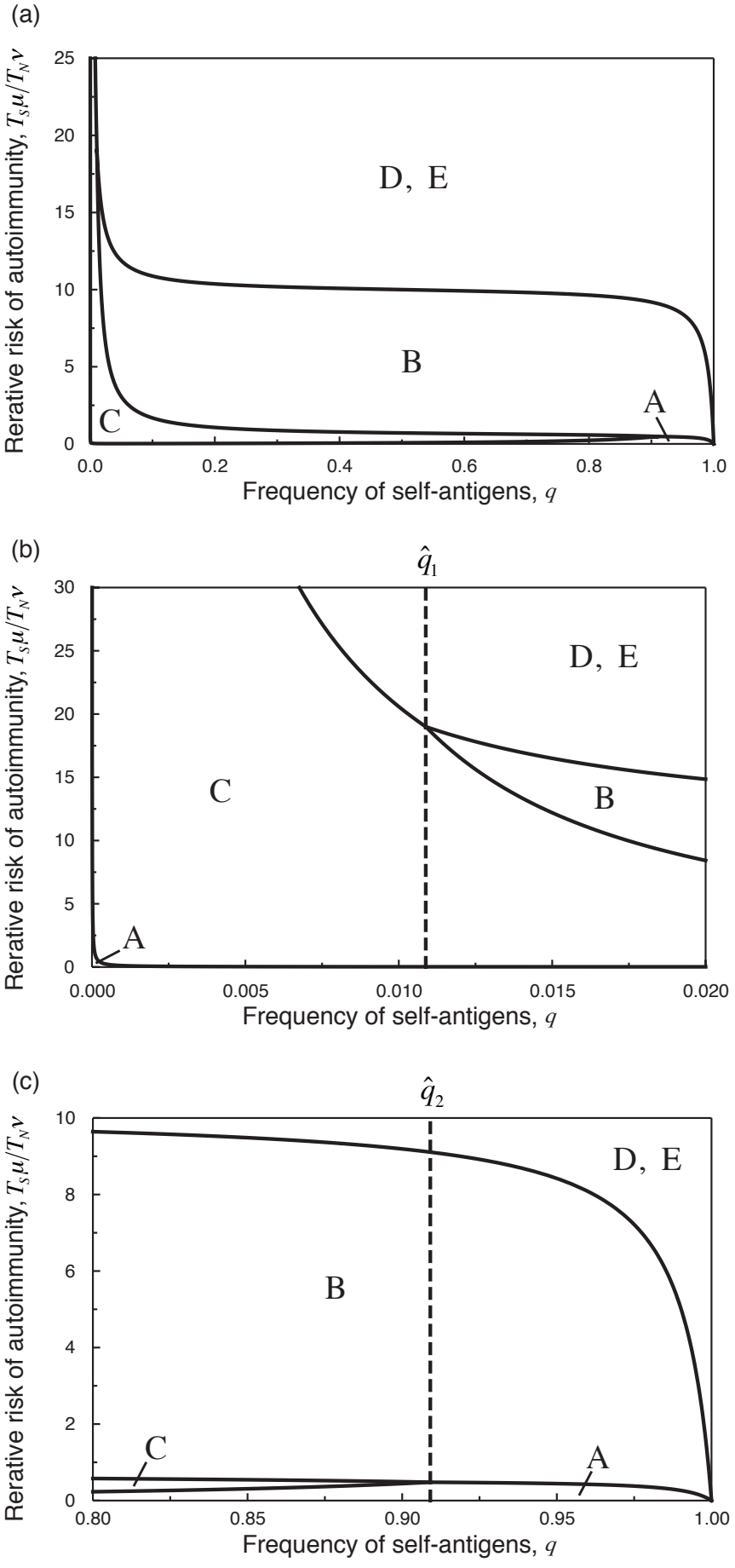

(a)
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
25
20
15
10
5
0
D, E
B
C
A
0.0
0.2
0.4
0.6
0.8
1.0
Frequency of self-antigens, q
(b)
$\hat{q}_1$
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
30
25
20
15
10
5
0
D, E
C
B
A
0.000
0.005
0.010
0.015
0.020
Frequency of self-antigens, q
(c)
$\hat{q}_2$
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
10
8
6
4
2
0
D, E
B
C
A
0.80
0.85
0.90
0.95
1.00
Frequency of self-antigens, q

Figure 3. 3

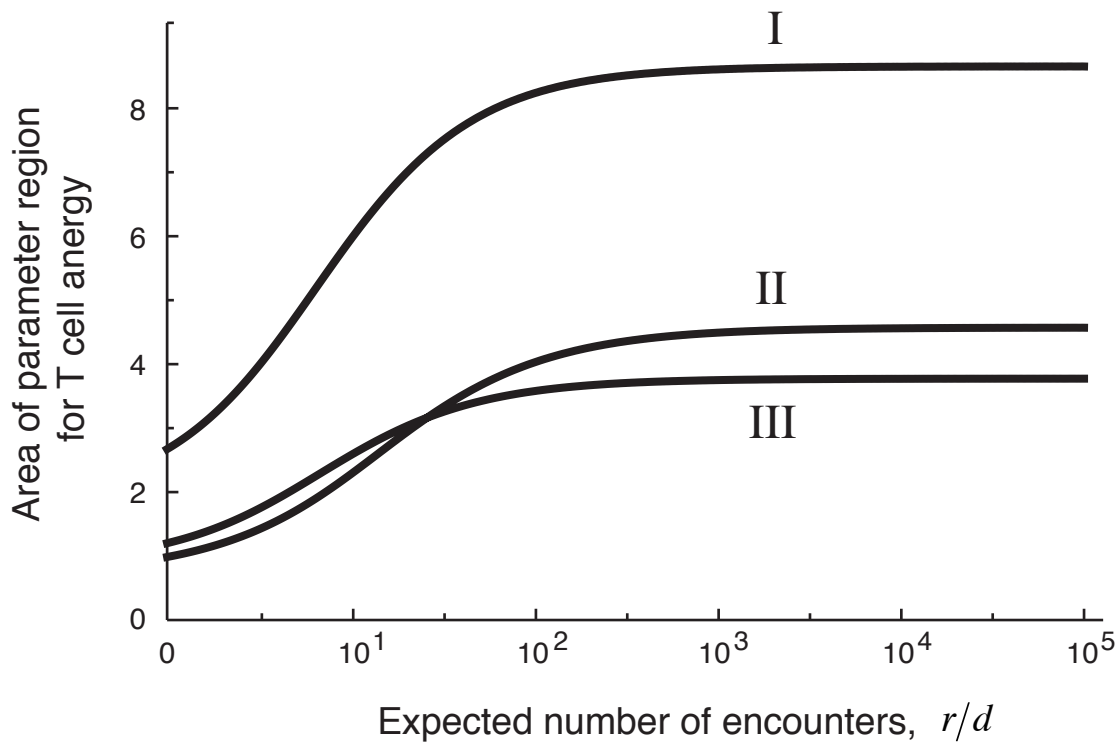

I
II
III
Area of parameter region
for T cell anergy
0
2
4
6
8
0
10^1
10^2
10^3
10^4
10^5
Expected number of encounters, r/d

Figure 3. 4

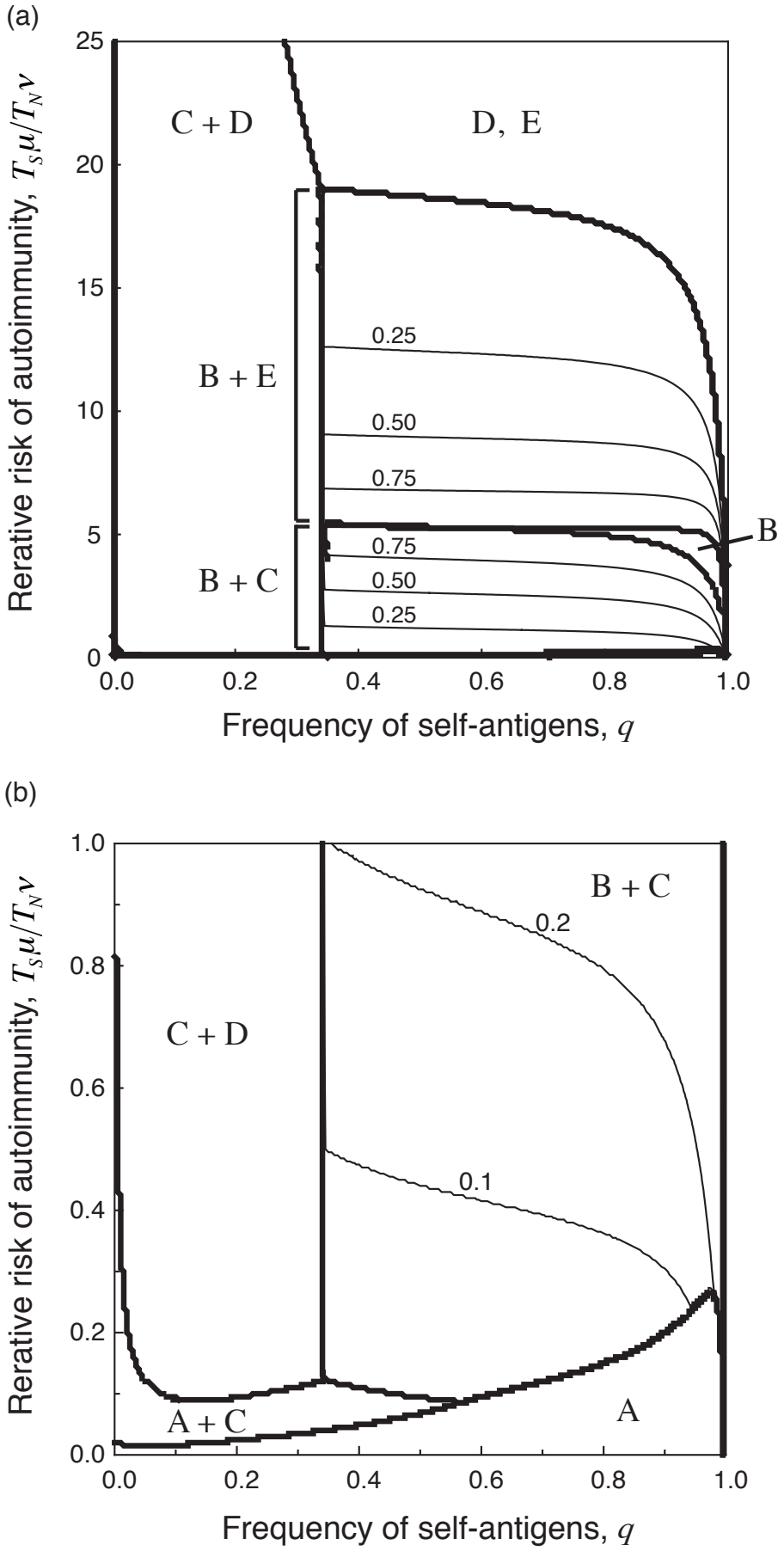

(a)
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
C + D
D, E
B + E
B + C
0.25
0.50
0.75
B
0.75
0.50
0.25
Frequency of self-antigens, q
(b)
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
B + C
0.2
C + D
0.1
A + C
A
Frequency of self-antigens, q

Figure 3. 5

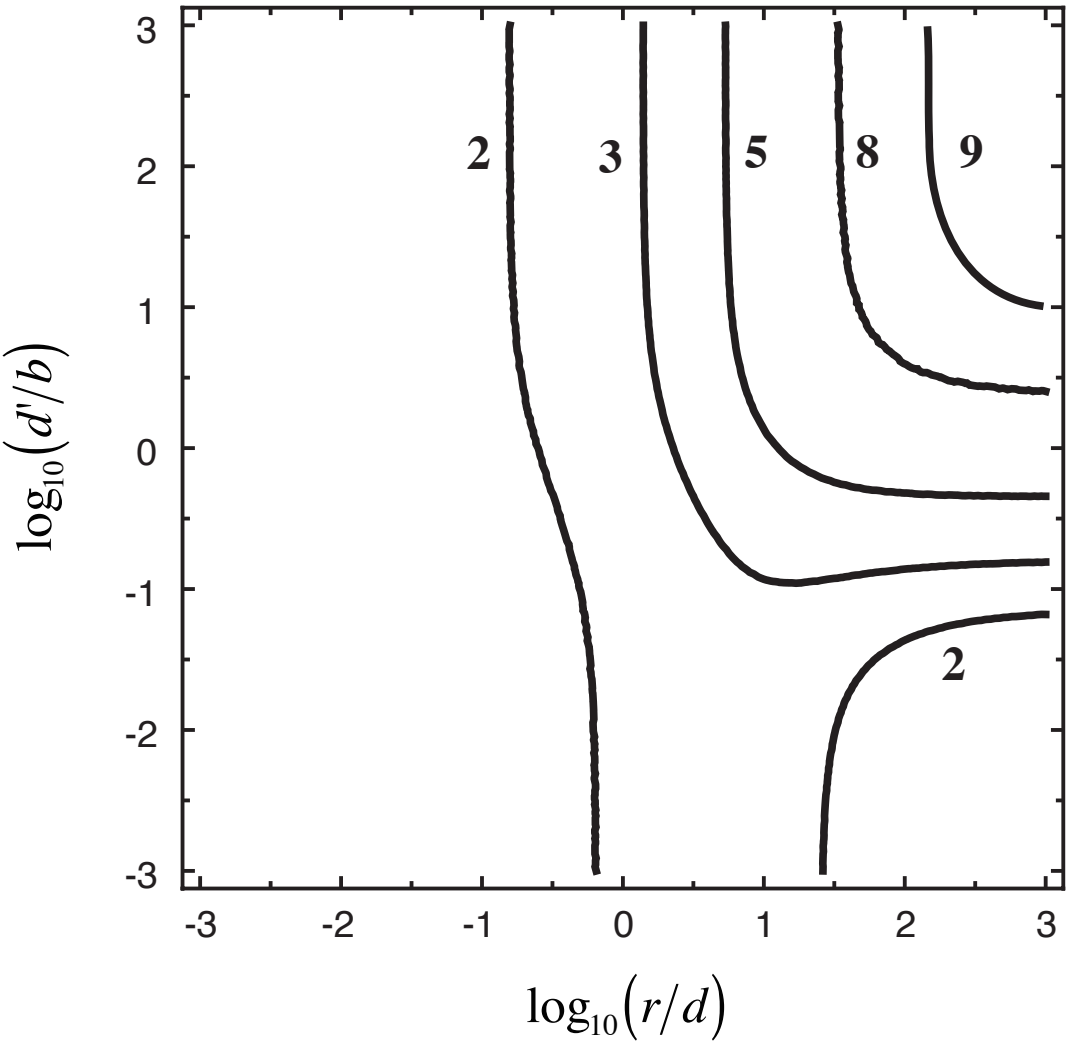
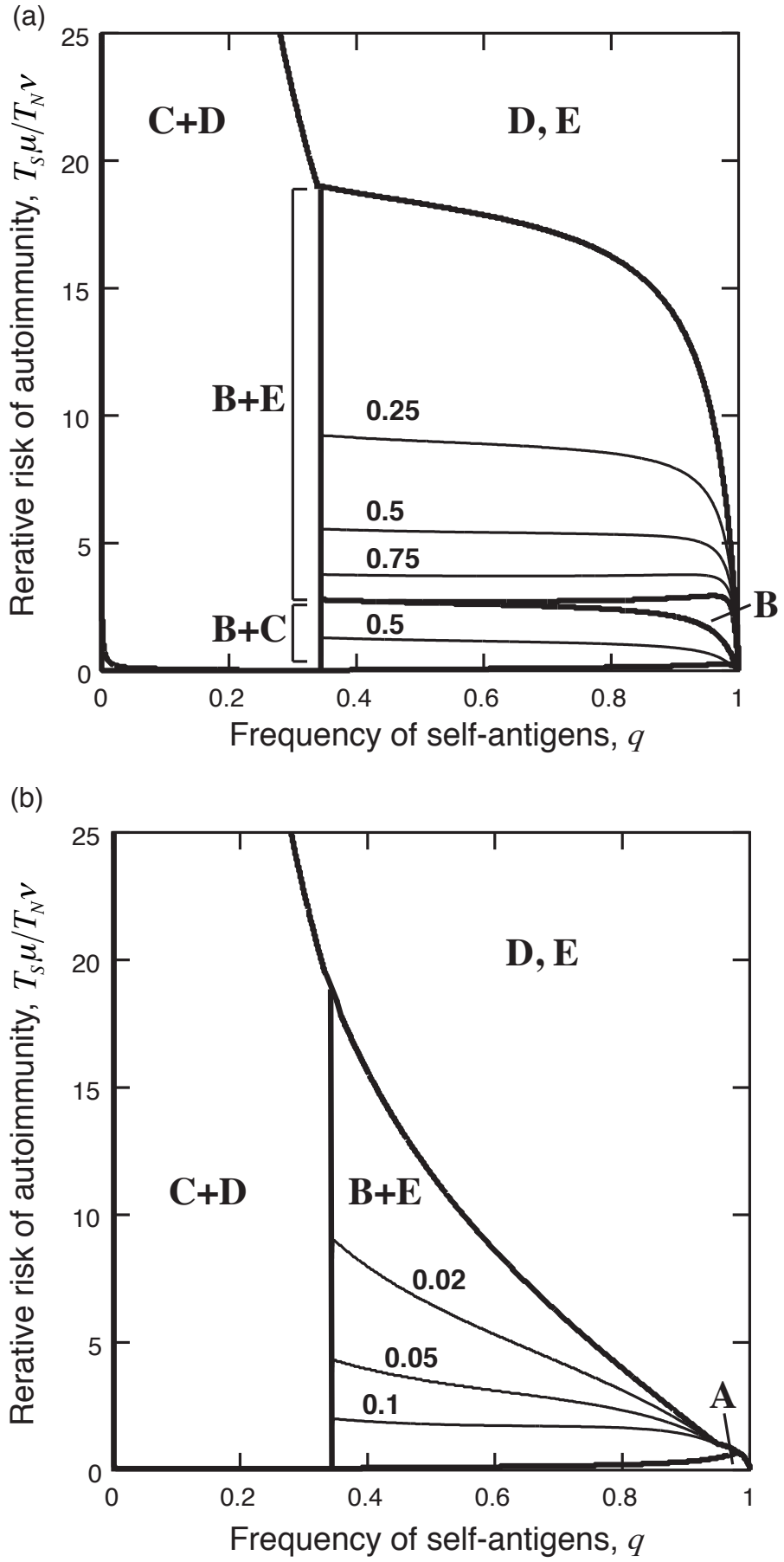


Figure 3. 6


(a)
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
C+D
D, E
B+E
B+C
0.25
0.5
0.75
0.5
B
0
5
10
15
20
25
0
0.2
0.4
0.6
0.8
1
Frequency of self-antigens, q
(b)
Rerative risk of autoimmunity, $T_S\mu/T_N\nu$
D, E
C+D
B+E
0.02
0.05
0.1
A
0
5
10
15
20
25
0
0.2
0.4
0.6
0.8
1
Frequency of self-antigens, q

Acknowledgements

I would like to appreciate my supervisor Prof. Yoh Iwasa for his advice, support, and invitation to the scientific world. My projects in this thesis were encouraged by him. I also thank to Prof. Takehiko Sasazuki, Prof. Akira Sasaki, Prof. Akihiko Yoshimura, Prof. Sho Yamasaki, Prof. Shingo Iwami, Dr. Yoshihiro Morishita, Dr. Shinji Nakaoka for their useful advices and comments. Especially, Prof. Rob de Boer patiently listened my clumsy English and gave me a fruitful discussion, and Prof. Yusuke Yanagi taught guided me to the principles of virology and immunology.

I'd like to show my gratitude extends to the present and previous members of mathematical biology laboratory, Kyushu University. They gave me an enjoyable and stimulable daily life. Finally, I also thank to my family, especially my parents Testuyoshi and Eiko for their great support and encouragement.