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Charlotte Hui Wang 04/01/2021

T Cell Priming Through Serial Encounters Lowers the Probability of Autoimmunity against DC-self

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Abstract

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During T cell development, T cells are clonally deleted by negative selection in the thymus in order to provide tolerance against self-reactive lymphocytes. The large number of possible self-peptide MHCs (spMHCs) and the relatively short period for negative selection in the thymus impose a quantitative constraint on the efficiency of clonal deletion against self-reactive lymphocytes. It is, however, particularly important to delete T cells specific for dendritic cell endogenous sp-MHCs – this is because DCs play a central role in the activation of T cells and the generation of immune responses. Since DCs in the thymus have very similar patterns of gene expression as DCs in the periphery, Matzinger has hypothesized that it may be possible to reliably delete all T cells specific for DC-endogenous sp-MHC (DC-self). Here we use quantitative models to explore Matzingers hypothesis. We begin with a simple model where the rules for stimulation of a T cell in the thymus is the same as that in the periphery. In this scenario, we find that it may be difficult to reliably delete T cells specific for rare DC-self. We then explore how differences in the dynamics of T cell activation in the thymus vs. the periphery may allow us to reliably avoid stimulation of T cells against DC-self. Our results suggest that the complex pattern of T cell priming in secondary lymphoid organs may arise, at least in part, to prevent the stimulation of autoimmune T cells responses to DC-self.

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Charlotte Hui Wang

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Chapter 1

Introduction

Biological systems have evolved intricate designs to maintain homeostasis during the course of an infection. For example, the mammalian immune system distinguishes between potentially harmful foreign antigens and non-threatening self-antigens and responds appropriately.

Each individual has a large number of lymphocyte clones, each expressing a different antigen receptor in order to provide immune protection against the rapidly evolving pathogens[1]. Effectively, during T cell maturation, genetic recombination of V(D)J gene segments of the T cell receptor (TCR) genes occur to create a large TCR repertoire that recognizes short peptides about 10 amino acids in length bound to one of several possible major histocompatibility (MHC) molecules expressed on the surface of the relevant host cells[2]. Recent estimates suggest that T cells have a potentially enormous repertoire of over 10^{15} possible TCRs, numbers that vastly exceed the number of T cells in a vertebrate[3,4]

While T cells make adaptive immunity possible, it is important to prevent T cell reactivity against self-antigens to avoid autoimmunity. The main mechanism for immune self-tolerance, the ability for the immune system to recognize self-antigens as non-threatening is clonal deletion, also known as central tolerance. The clonal deletion theory posits that tolerance is achieved by the deletion of self-reactive lymphocytes during their ontogeny[5].

For T cells maturing the thymus, T cells with very low and high affinities for self-peptide MHCs (sp-MHCs) of antigen presenting cells are deleted. Deletion of cells with very low affinity for spMHC eliminates useless T cells which are incompatible with self-MHCs, and deletion of cells with high affinity for spMHC eliminates T cells that will potentially cause autoimmunity[6,7].

1.1 Central Tolerance and Its Limitations

However, there are limitations to the effectiveness of central tolerance. Work in this paper builds on the study of Muller and Bonhoeffer, as they propose there is a quantitative constraint on the scope of negative selection, also known as central tolerance. They propose that central tolerance is not sufficient for a number of reasons. 1). Not all self-antigens are expressed by APC in the thymus[8]. 2). There may not be sufficient time for T cells to reliably scan through all sp-MHCs presented by APCs during negative selection in the thymus[9]. Moreover, Müller and Bonhoeffer's model construction takes the perspective of an antigen presenting cell (Appendix A). Their estimation of the efficiency of negative selection is based on the assumption that antigen presenting cells uniformly express peripheral tissue antigens (PTA), which is an assumption now challenged by much recent research in the medullary thymic epithelial cells and their roles in PTA presentation under the regulation of the autoimmune regulator (AIRE)[9,10]. Therefore, we wish to take on the perspective of a potentially autoreactive T cell and improve from their estimations. This new perspective considers a T cell's phenotypic change in response to stimuli, rather than looking at antigen presentation capabilities of DCs. More theoretical work in the field tried to quantify thymic selection and understand the generation of the T cell receptor post thymic selection and they all acknowledge that selection is not complete in deleting all self-reactive T cells because these potentially self-reactive T cells are important in generating a complete immune repertoire to protect against foreign infections[7].

Empirically, researchers have found evidence of antigen-specific T cells escaping thymic selection[11,12]. With a rip-mOva transgenic mouse system, Enouz et al. detected Ova specific T cells that escaped thymic selection and circulated in the periphery. Though only lower avidity T cells specific for the cognate antigen escaped, if stimulated under the right conditions, researchers have shown that low avidity self-reactive T cells can elicit an autoimmune response such as causing T cell attack on pancreatic beta cells and increase blood-glucose levels of rip-mOva mice[12]. Researchers have quantified the level of affinity of Ova peptides required for a T cell to escape thymic selection and have inferred that thymic selection is only efficient above a certain TCR affinity threshold[13]. Additionally, other empirical results have shown that 25%-40% of self-reactive T cells with intermediate to low affinities for the cognate antigen escape clonal deletion[14]. From this evidence, we recognize that in order to quantitatively understand the mechanisms underlying immune tolerance, estimating the efficiency of central tolerance is not sufficient. Lower avidity self-reactive T cells may escape thymic selection and elicit autoimmune responses.

Since we aim to study autoimmunity generated by peripheral activation of self-reactive T cells and autoimmune attack of self-tissues, we will focus on CD8 T cells from now on.

1.2 Peripheral Tolerance Mechanisms

There are many peripheral tolerance mechanisms in place to prevent autoimmunity. For example, the initiation of immune responses – clonal expansion of T cells specific for a given peptide presented by MHC – requires the T cells to receive two signals from an antigen presenting cell (in this case, dendritic cells). The first, termed signal 1 or antigen-specific stimulation, is the stimulation of the TCR by the peptides bound to the MHC on the surface of DC. The second, signal 2 or co-stimulation (i.e. CD28), requires the activation of a DC which occurs when the DC senses the presence of pathogens or infection (also called danger

signals)[8]. There is experimental evidence showing that when a T cell encounters a cognate antigen in the absence of the costimulatory molecule, it becomes anergic—intrinsically inactive – and does not elicit immune responses[15,16]. As a result, it is important to understand how the anergic state of T cells contributes to the overall prevention of autoimmunity. Some theoretical biologists such as Cliburn Chan et al have attempted to include anergic states of T cells in the quantification of autoimmunity[17]. (see more in Appendix B)

Peripheral tolerance mechanisms that prevent autoimmunity can be divided into two categories: T cell intrinsic mechanisms and T cell extrinsic mechanisms. The first category encompasses tolerance mechanisms that are generated by T cell properties and phenotypic changes, whereas the latter category includes tolerance mechanisms that involve other immune cell types and their interactions with T cells. For example, the generation and activities of regulatory T cells are T cell extrinsic peripheral tolerance mechanisms that we will not explicitly address in our model here[18]. T-cell intrinsic peripheral tolerance mechanisms include activation-induced apoptosis, the deletion of self-reactive T cells under the regulation of the Fas ligand and its receptor[19,20,21]. It is apparent that T cells make intrinsic cellular decisions upon environmental stimuli, thus before we discuss peripheral tolerance mechanisms, it is important to first understand how T cells make cellular decisions such as activation in the periphery. Fundamentally, it is important to elucidate how T cell signaling cascades are activated and regulated by TCR and pMHC binding.

1.3 T cell activation requirements

In the periphery, two-photon microscopy studies of lymphocyte activities in secondary lymphoid organs have shown that primary T cells require contact dependent information exchange between T cells and dendritic cells for priming and activation. Specifically, T cells initially undergo multiple short encounters with DCs, progressively decreasing mobility and upregulating the activation markers and then transitions into more prolonged

interaction to prepare for activation and proliferation[22]. Mempel et al., has characterized these events as a three stage activation requirement:

1. The T cells go through short-transient contacts (6min/contact), decrease their mobility and upregulation of activation markers (priming phase)
2. Followed by prolonged interactions (≈30 min/contact) and secretion of IL-2 and IF-γ (stable-contact phase)
3. Ending with another series of short DC contacts and rapid migrations (high motility and rapid proliferation)

Interestingly, it appears that T cells go through similar three stage behaviors even when DCs do not present the cognate antigen. Regardless of the antigen presentation, Mempel et al., have shown that a T cell spends just enough time in a lymph node to complete all three phases before leaving through efferent lymphatics. If, during the priming phase, the T cell did not receive the correct signals for proliferation, then the stable-contact phase (phase 2) is much shorter compared to when T cells did receive signals from DC presentations. The default activity of a T cell when it enters a lymph node is this three-stage series, and the difference in activation vs. continuing migration is dependent on the priming phase.

Meanwhile, Faroudi et al. have independently shown that intermeditted signaling without a stable immunological synapse can commit T cells to cytokine productions suggesting that T cell activation may result from summation of interrupted signals[23].

Researchers have proposed that the mechanism in which T cells regulate responses after DC encounters, is based on an integrated signal that tabulates successful encounters until a threshold is met[24,25]. Understanding how T cells are activated upon a series of encounters is a key function of T cells to consider in our quest of quantitatively understanding how auto-reactive T cells respond to signals appropriately in mammalian systems. Moreover, it was shown experimentally that T cell activation is a stochastic process, which allows for probabilistic modeling of T cell dynamics[26].

1.4 Our Focus

For our model constructions, a specific subset of CD8 T cells– the subset of dendritic cell self-peptide specific T cells – was suggested to be particularly important for quantitative estimations:T cells specific for DC endogenous peptides (DC self). This is because:

1. DCs are important in presenting antigens and costimulatory signals to T cells, consequently it is essential to delete T cells against self-peptides endogenous for DC (DC-self) in order to ensure appropriate immune activation.
2. DCs are constantly present during T cell activation in the periphery, thus the probability of T cells self-reacting against DC-self is high if they are not deleted in the thymus.
3. DCs have similar gene expression in both the thymus and periphery, unlike tissue-restricted proteins which are only briefly expressed during negative selection in the thymus.
4. Based on Dr. Polly Matzinger's hypothesis, since DCs are so important in immune function and have similar gene expressions in both the thymus in the periphery, central tolerance must be sufficient and efficient in deleting all T cells specific for DC-endogenous sp-MHC (DC-self) 1994. In other words, it may be possible to reliably delete all T cells specific for DC-self. We wish to use quantitative models to evaluate whether or not central tolerance is robust in this framework, and if not, what other mechanisms are in play.

In the context of DC-self specific T cells, we aim to use quantitative models to estimate how successful T-DC interactions affect the outcome of self-reactive T cells. We hypothesized that the combination of central tolerance with empirically observed signal integration of TCR signals at subthymic thresholds can prevent autoimmunity against DC-self.

Chapter 2

Model 1- Single-hit model

In the simplest scenario considered for a mathematical modeling of T cell tolerance, we assume that the rules of stimulation of a DC-self specific T cell upon encountering sp-MHCs on a DC should be the same in the thymus and the periphery, given the similar patterns of gene expression. The probability of a T cell escaping the thymus is a key indicator of successful generation of tolerance, but we also acknowledge that successful generation of tolerance also encompassess the probability of an escaped autoreactive T cell to not be stimulated in the periphery.

Here we evaluate the problem of tolerance against DC-self by considering both the probability of a self-reactive T cell escaping the thymus and the probability of it being stimulated in the periphery, together defined as the probability of autoimmunity. The only MHC class that is relevant here is the MHC class I sp-MHCs, because we are focusing on the deletion of CD8 T cells (endogenous DC self-peptides are presented on the MHC class I) and the activation of CD8 T cells.

2.1 Model construction

Table 1 lists all the parameters relevant for our simple probabilistic model for T cell dynamics in the thymus and the periphery.

Table 1: Model 1 Parameters

Abbreviation	Description	Values
p-MHC	peptide presented on MHC	
sp-MHC	self-peptide presented on MHC	
DC-self	self-peptides presented on *all* DC	
p	Probability of signaling (TCR binding) per encounter (Signal 1)	range [0,1]
$p_1 = p$	Probability of T cell clonal deletion per signal 1 encounter in the thymus	range [0,1]
$p_2 = p/k$	Probability of T cell activation per signal 1 encounter in the periphery	$k \geq 1$
k	Difference factorDifference in probability of apoptosis in thymus vs. actiation in the periphery	$k \geq 1$
n_1	number of DC encounters in thymus	4000
n_2	number of DC encounters in periphery	n_2 varies

Let p be the probability of signal 1 and measured by the avidity and affinity of a TRC against an sp-MHC complex. In our simple models, signal 1 leads to either clonal deletion in the thymus (p_1) or T cell activation in the periphery(p_2) following an encounter with a DC for a DC-self reactive T cell. p takes into account factors such as both the 1. level of expression of the relevant DC-self peptide, and 2. the affinity of the TCR for the peptide, and it can range from 0 to 1. If this T-cells has n_1 encounters with DC in the thymus then the probability that the cell escapes depletion in the thymus is:

$$\text{probability of escape in thymus} = P_E = (1 - p_1)^{n_1}$$

If the T cell has n_2 encounters with activated DC in the periphery then the probability of activation in the periphery is:

$$\text{prob. of stimulation in periphery} = P_S = 1 - \underbrace{(1 - p_2)^{n_2}}_{\text{prob. of no stim. in } n_2 \text{ trials}}$$

and for autoimmunity to occur the T cell must escape deletion in the thymus and be stimulated in the periphery[17].

$$\text{prob. autoimmunity} = P_A = P_E \times P_S = \underbrace{(1-p_1)^{n_1}}_{\text{prob. of no stim in thymus}} \times \underbrace{1-(1-p_2)^{n_2}}_{\text{prob. of stim in periphery}}$$

Based on previous quantitative estimates of the amount of T-DC contacts during negative selection, we chose $n_1 = 4000$ similarly to Müller and Bonhoeffer[9]. The amount of T-DC encounters in the periphery, denoted by n_2, is hard to quantify depending on the context of the T cell condition we are assessing. Moreover, the factor k equals the difference in probability of thymic selection in the periphery and the probability of peripheral stimulation ($p_2 = p/k$). In our simple consideration, we set the requirements for clonal deletion the same as the requirements for peripheral stimulation, thus k=1. And we proposed that it is harder for T cells to be stimulated in the periphery than it is for T cells to be deleted in the thymus, thus $k \geq 1$.

2.2 Model results

Under a single-hit model, we see that under similar conditions of clonal deletion and peripheral T cell activation, low affinity/avidity T cells (low p) have the highest probability of escaping thymic selection–The probability of an autoreactive T cell escaping negative selection in the thymus (red) decreases as log p is greater than -3 (Figure 1).

High affinity/avidity T cells (high p) are very unlikely to escape thymic selection, thus will not cause a problem for autoimmunity. However, The probability of autoimmunity (blue) increases as logp (probability of signal 1) approaches -3, and the probability of autoimmunity drastically decreases as logp is within greater than -3: the probability of stimulation of a autoreactive T cell in the periphery increases as logp increases to an intermediate level. T cells that fall into this intermediate range of affinity/avidity are the potentially self-reactive

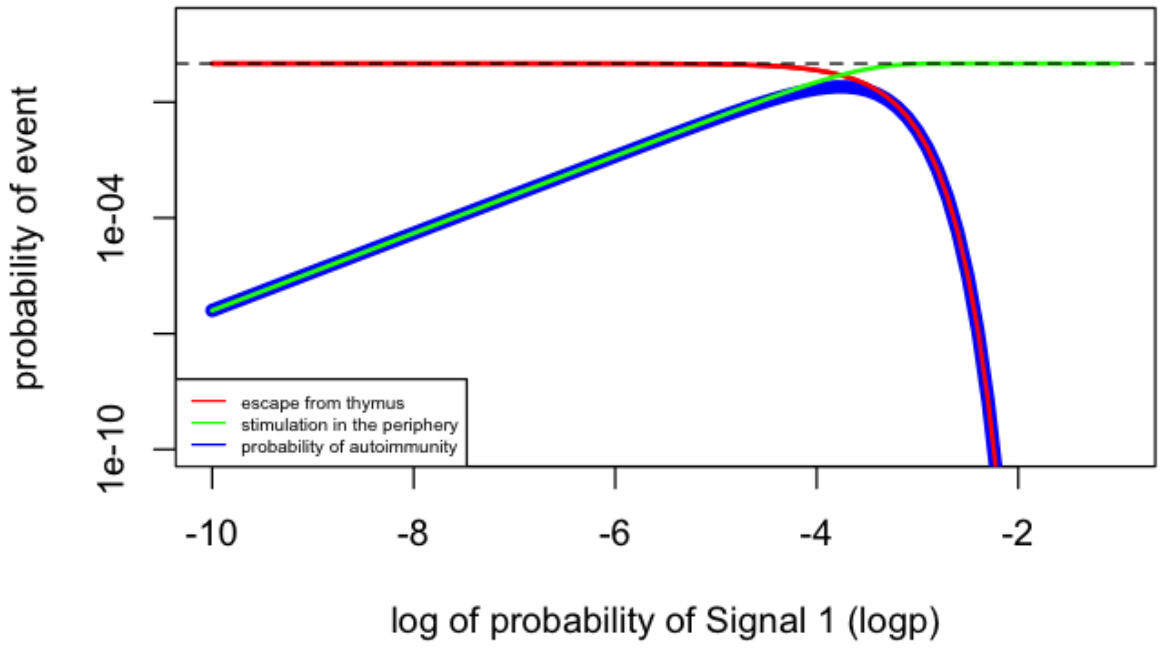


Figure 1: **The probability of T cell induced autoimmunity depends on the probability (p) of thymic escape and peripheral activation.** Thymic escape (red) and T cell stimulation in the periphery (green) contribute to T cells with intermediate affinity and avidity having the highest probability of causing autoimmunity (non-monotonic blue curve). Under a simple model, the rules for T cell stimulation in the periphery is the same as the rules for T cell clonal deletion in the thymus. Referencing the probability of 1 (dashed line), intermediate affinity/avidity T cells (intermediate p) have the highest probability of escaping the thymus (red line) after 4000 DC encounter but a very low probability of being stimulated in the periphery (green line) after 4000 DC encounters. On the other end, very high affinity/avidity T cells (high p) are extremely unlikely to escape the thymus (red line), so although these T cells would likely be stimulated if they escaped the thymus (green line), they are unlikely to cause problems (blue line) ($p_1 = p_2$, $k = 1$, $\tau = 1$, $\alpha = 1$, $n_1 = 4000$, $n_2 = 4000$)

T cells that can escape thymic selection and may lead to autoimmunity under the right stimulation conditions[12]. The maximum probability of autoimmunity at this intermediate rage of DC self-reactive T cells ($p = 10^{-3.6}$) is 5%.

2.2.1 Changing n_2

Initially, under the same rules and conditions for thymic selection and peripheral activation, the number of DC encounters in the periphery was set as the same as the number of DC encounters a T cell makes in the thymus ($n_1 = n_2 = 4000$). However, this does not depict biological realities. During the time-span a T cell spends in one lymph node, T cells encounter ≈40-80 different DCs[22], thus we chose an n_2 of 60 to evaluate the probability of autoimmunity during the T cell's first lymph node scanning after it escapes the thymus. This choice of $n_2 = 60$ was set as our lower bound for n_2. The maximum probability of autoimmunity when $n_2 = 60$ is 5%. But we then use different n_2 values to understand this change in probability during the lifetime of a T cell. As n_2 increases, the probability of autoimmunity increases (Figure 2). Moreover, the lifetime of a T cell is 3 years, thus we chose $n_2 = 200,000$ as our upper bound, which corresponds with a maximum probability of autoimmunity 90.6%. There is no empirical evidence of autoimmunity disease against dendritic cells so we need to further evaluate the effects of different mechanisms that must be in play to prevent autoimmunity. By approximating the maximum probability of autoimmunity, we see that the change in n_2 is directly proportional to the change in maximum probability of autoimmunit (Appendix C).

2.2.2 Changing Factor k

Factor k, which is the difference factor ($p_2 = \frac{p_1}{k}$), equals the differences in the probability of signaling in the thymus leading to clonal deletion vs. probability of signaling in the periphery leading to T cell activation. A successful signal 1 stimulus leads to very different biological outcomes in different environments, thus our first passage of quantifying

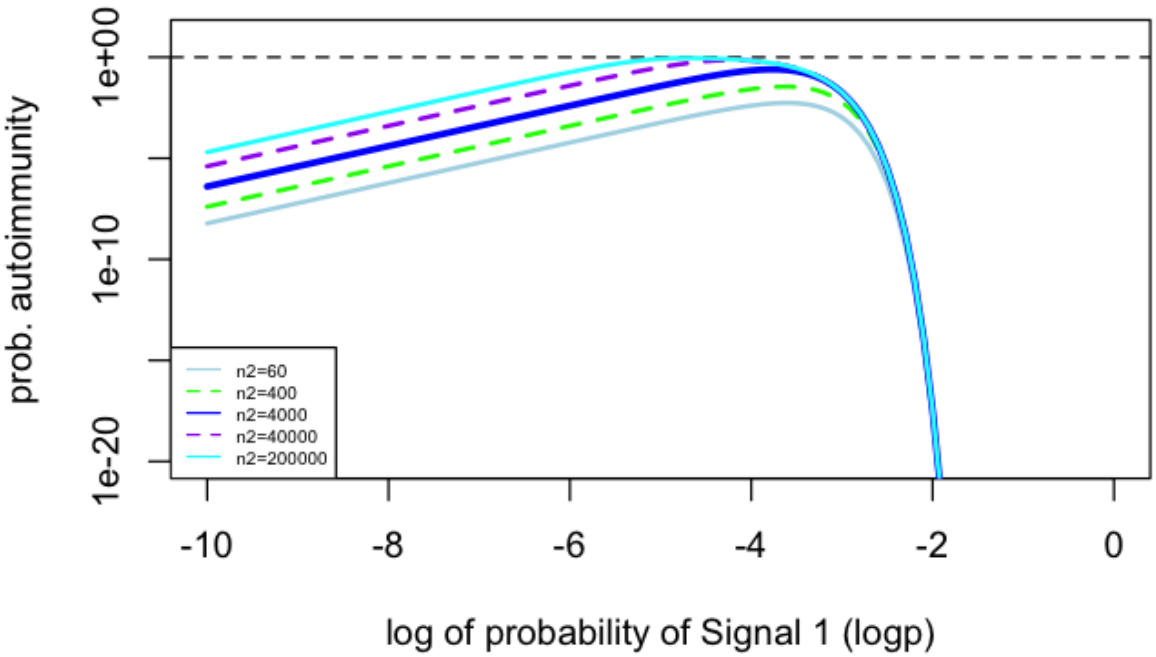


Figure 2: **The probability of autoimmunity increases as the number of DC encounters in the periphery (n2) increases.** In the periphery, a T cell encounters >> 4000 DCs during its lifespan, thus n2 > n1. During a lifespan (n2 = 200,000) of potentially self reactive T cells, the highly reactive ones (p > 1e-2) will be eliminated by central tolerance and the poorly self reactive ones (p < 1e-6) are less likely to get stimulated. The T cells with intermediate range of affinity/avidity to DC-self can reach as high as 90% probability of autoimmunity if not regulated during the lifetime of a T cell (cyan solid line). During one lymph node encounter, (n2 = 60), the maximum probability of autoimmunity for the intermediate avidity T cells is 5% (red solid line). Taken together, other mechanisms and complexities of the immune system's T cell activation rules have to be assessed. (p2 = p1/k, k = 1, $\tau = 1$, $\alpha = 1$, n1 = 4000, n2varies)

the difference in the dynamics of T cell response upon a successful signal 1 encounter is achieved by changing the value p takes on in the thymus vs. in the periphery.

$$p_2 = \frac{p_1}{k}$$

Thus,

$$\text{prob. autoimmunity} = P_A = P_E \times P_S = \underbrace{(1-p_1)^{n_1}}_{\text{prob. of no stim in thymus}} \times \underbrace{1-(1-p_1/k)^{n_2}}_{\text{prob. of stim in periphery}}$$

From changing k, we observed that changing k does decrease the probability of autoimmunity. Increasing k changes the rules and phenotypes of T cells in the thymus and the periphery, and makes it harder for a T cell to be stimulated upon signal 1 encounter in the periphery, than it is to be clonally deleted upon signal 1 encounter in the thymus (Figure 3), quantified by TCR avidity/affinity against sp-MHC (p). Based on the approximation of the maximum probability of autoimmunity, we see that the change in k is inversely proportional to the change in probability of autoimmunity (Appendix C).

This simple model demonstrates that while central tolerance against DC-self may be sufficient to prevent autoimmunity against high avidity antigens, T cells with an intermediate avidity for DC-self ($10^{-4} < p < 10^{-3}$) are likely to both escape negative selection in the thymus and trigger autoimmunity in the periphery (Fig 1). However, we do not empirically observe high levels of autoimmunity against DCs.

Different mechanisms may affect the stochastic, low probability phenomenon of T cell activation against DC-self in the periphery. With the low T cell affinities/ avidities that we consider ($10^{-4} < p < 10^{-3}$), a modeling approach is useful to identify the biological mechanisms to which T cell activation and autoimmunity are most sensitive. We then evaluated a set of computational models that explicitly considers the different rules and requirements for peripheral T cell activation vs. thymic selection.

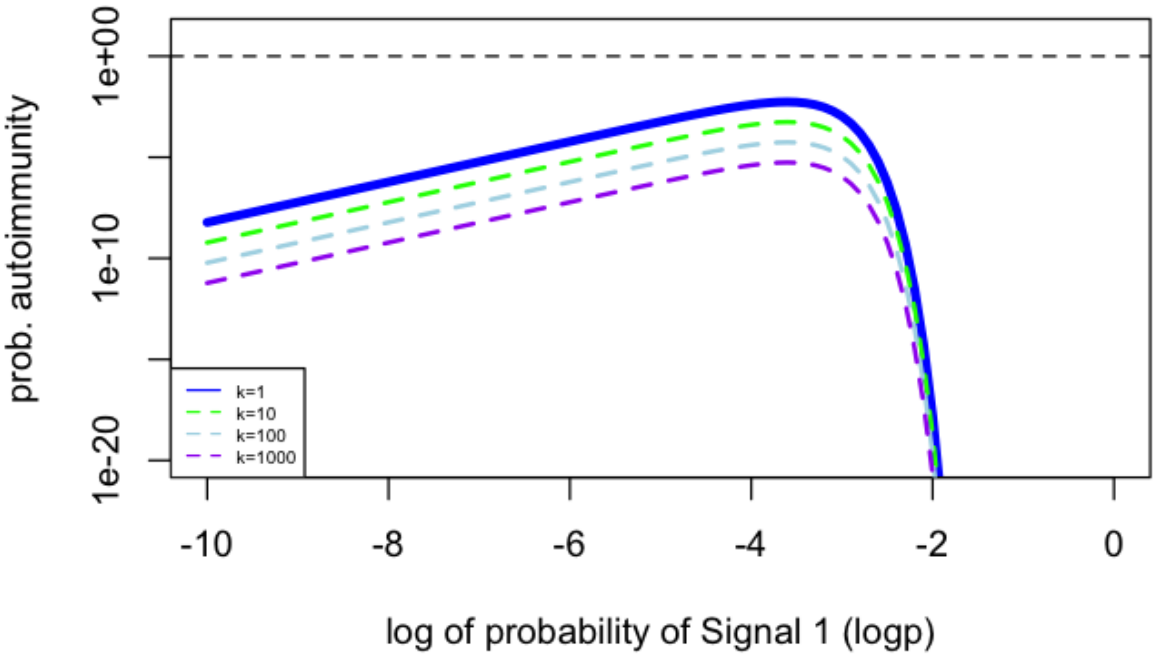


Figure 3: **The probability of autoimmunity decreases when the T cell has a higher threshold for stimulation in the periphery compared with the thymus.** Factor k equals the difference in the rules of clonal deletion in the thymus vs. the rules of activation of a T cell in the periphery. As we increase k to 1000 fold, indicating a 1000 fold decrease in p_1(thymus) vs p_2 (periphery), it is harder for a T cell to be stimulated in the periphery considering one local lymph node and only 60 encounters with DCs. In this case, it is also harder (1000x) for a self-reactive T cell to cause autoimmunity. As shown in the Appendix, the maximum probability of autoimmunity is inversely proportional to k (Appendix 3). ($p_2 = p_1/k$, $kvaries$, $\tau = 1$, $\alpha = 1$, $n_1 = 4000$, $n_2 = 60$)

Chapter 3

Model 2- Serial Encounter Model

From the results of model 1, we see that the probability of autoimmunity is very high for rare antigens (when $p \approx 10^3$ for DC-self) given those specified T cells have high probabilities of stimulation per encounter. We now consider the differences in the results of TCR binding (signal 1) between the thymus vs. the periphery. We incorporate the different stages of priming of a T cell in the periphery may help us modify our model. Two-photon microscopy studies of T cell-DC interactions for the duration of homing to lymph nodes suggested that T cells make integrated "measurements" over the course of multiple serial encounters with different DCs (≈6 min/contact for ≈4-8 hours). Further studies have shown that T cells accumulate these transient weak signals over time until a threshold is met to commit to proliferation[27].

3.1 Model construction

We hypothesize that the multiple encounters of DCs necessary for priming produce multiplicative signals that restrict the probability of stimulation upon encounter. We hypothesize that every successful encounter will increase the signal by an additive signal manipulator constant of α, and once the signal exceeds a threshold τ, then the T cell will proliferate (stimulated). Meanwhile, Mempel et al., has shown that a T cell is constantly

migratory. If it is not stimulated after the priming phase of one lymph node, then the T cell migrates to another lymph node and starts the priming process all over again.

We used a probabilistic model, modeling the probability of proliferation using factors such as the number of serial encounters, the increasing signal factor per every successful encounter, and the probability of stimulation per encounter at the periphery. In our model, time is a discrete variable and we assume that one time interval is one T-Dendritic cell interaction.

Computationally, if the encounter is successful (determined by a randomly generated probability compared to p), then the integrated signal (S_i) increases by a ($+a$) (Algorithm 1). When the integrated signal (S_i) reaches the threshold τ, then a T cell is activated. This turns out to be a binomial model in which we can calculate the combined probability density of a binomial distribution function given a certain probability of successful encounters (p) (Algorithm 1 execution, p.nd function). We chose $n_2 = 60$ for the number of encounters in the periphery. Biologically, this is the approximate number of T-Dendritic cell encounters during one lymph node priming phase (Table 2). This is similar to a biological local acute infection, and we assume that all DCs that the T cells encounter are activated (DCs express costimulatory molecules such as CD80), thus signal 2 is present during all DC encounters. By using this small n_2, we are assuming that the memory of integrated signals is cleared if the T cell is not stimulated in one lymph node– the signal accumulation restarts once the T cell enters another lymph node. This assumption is in place to allow us to understand what happens during one T cell priming series of one lymph node, and obtain an upper bound to the level of self-reactivity and potential probability of autoimmunity of the system.

Moreover, T cell's serial encounter of dendritic cells and integrated signals have shown to not only happen in the periphery, but also in the thymus[28]. The notion that T cells have to make serial encounters with DCs before determining its fate and responding appropriately via TCR signals is a conserved process in T cell phenotypes, and the outcomes

of such a process differs depending on the environment the T cells are in. For simplicity, we note that the rules and signals are different in the thymus vs. in the periphery, thus we assumed that $\tau = \alpha = 1$ in the thymus, indicating that a successful Signal 1 encounter (TCR binding) leads to enough signal accumulation to determine cell fate– in the thymus, the cell fate of correct TCR binding (p) is cell death (clonal deletion). In this case, the computational model of serial encounters and sequential clonal deletion simplifies to the original simple probabilistic model we had for calculating the probability of an autoreactive T cell escaping the thymus.

$$\text{probability of escape in thymus} = P_E = (1 - p_1)^{n_1}$$

Taken together, we now have the analytical solution to the probability of a self-reactive T cell escaping the thymus and a computational solution to the probability of a T cell being stimulated in the periphery (Algorithm 1 execution code). Together, we can calculate the probability of autoimmunity.

Algorithm 1: Serial Encounter Model- binomial model equation and execution

1

$$x_n \sim Bernoulli(p) \tag{3.1}$$

$$S_n = \begin{cases} S_{n-1}, & \text{if } x_n = 0 \\ S_{n-1} + a, & \text{if } x_n = 1 \end{cases} \tag{3.2}$$

$$P_s = P((\sum_{n=1}^{N} I(S_n \geq \tau)) \geq 1) \tag{3.3}$$

2 where

$$\tau = 1 \tag{3.4}$$

$$S_0 = 0 \tag{3.5}$$

$$n \in [1, N] \tag{3.6}$$

```
p.nd<-function(p, n2, a){
  return(1-pbinom(ceiling(1/a)-1, size=n2, prob=p))
}
```

3.2 Model results

In both single-hit and serial encounter models, T cells with higher affinity/avidity to DC-self are more likely to be stimulated in the periphery. In Figure 4, ss p increases, the probability of T cell stimulation (green) approaches 1 (referencing the black dashed line). However, these high affinity/avidity T cells will not lead to autoimmunity because they are deleted in the thymus (red). When the probability of thymic escape and T cell stimulation

Table 2: Model 2 Parameters

Abbreviation	Description	Values
p-MHC	peptide presented on MHC	
sp-MHC	self-peptide presented on MHC	
DC-self	self-peptides presented on *all* DC	
p	Probability of signaling (TCR binding) per encounter (Signal 1)	range [0,1]
$p_1 = p$	Probability of T cell clonal deletion per signal 1 encounter in the thymus	range [0,1]
$p_2 = p/k$	Probability of T cell activation per signal 1 encounter in the periphery	$k \geq 1$
k	Difference factorDifference in probability of apoptosis in thymus vs. actiation in the periphery	$k \geq 1$
α	magnitude of integrated signal per successful signaling event	$\alpha = seq(0, 1, 0.1)$
S_i	integrated signal value after ith encounter	range [0,1]
τ	threshold for stimulation (T cell activation occurs when $S_i \geq \tau$)	$\tau \geq 1$
n_1	number of DC encounters in thymus	4000
n_2	number of DC encounters in periphery	$n_2 = 60$

are combined, the probability of autoimmunity also decreases in a serial encounter model (blue solid line) compared to the single-hit model (blue dashed line) (Figure 4).

The maximum probability of autoimmunity is the highest probability of autoimmunity value for a specific set of thymic vs. peripheral conditions over a range of different p values. As the additive signal magnitude decreases, indicating that more successful encounters have to be made during the limited amount of DC encounters of the priming phase ($n_2 = 60$), the maximum probability of autoimmunity also decreases. However, under the presented model, if we relax the assumption that the integrated signal memory is cleared after each lymph node scanning, and allow the signal to accumulate indefinitely during a T cell's lifespan, the maximum probability of autoimmunity approaches 1 over time (Figure 5).

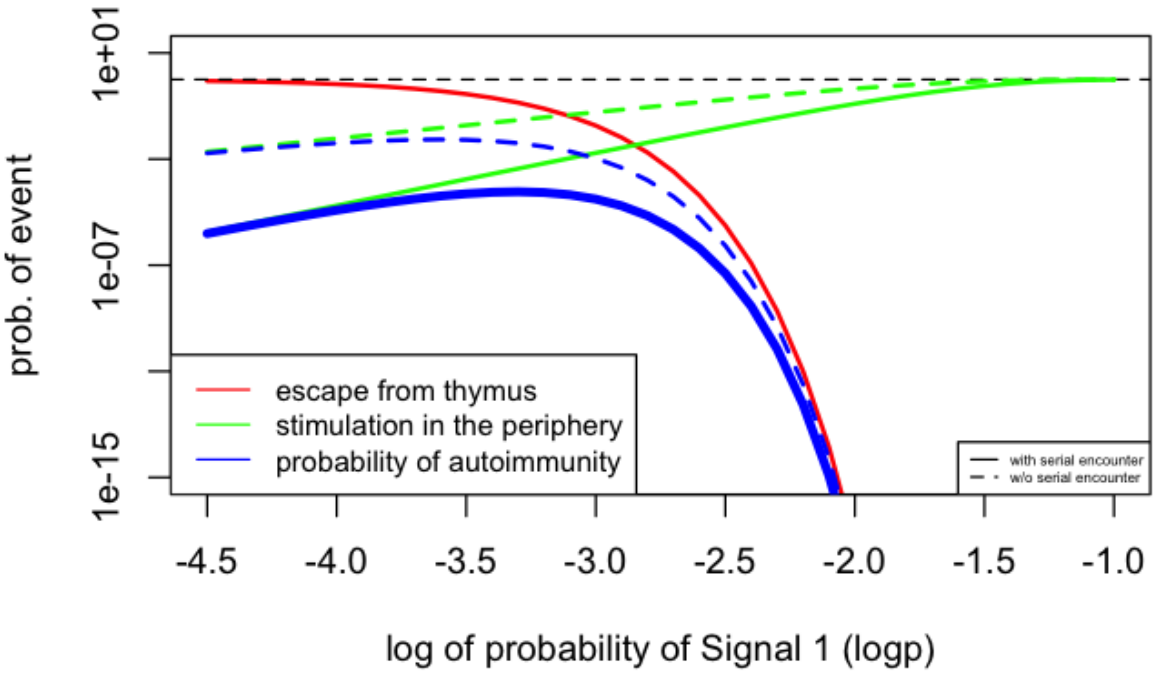


Figure 4: **Compared to the single-hit model (blue and green dashed lines), the serial encounter model (solid lines) decreases the probability of T cell stimulation in the periphery (green) and lowers the probability of autoimmunity (blue).** In model 1, T cells only needed one single productive encounter to be stimulated in the periphery. We examined the consequences of a stimulation requirement of multiple serial encounters. Since we simplified the rules of clonal deletion and set $\alpha = 1$ for the thymus, the probability of thymic escape (red) after 4000 DC encounters in the thymus does not change with serial encounter. We consider the scenario of a T cell peripheral stimulation during the timeframe it spends in one lymph node (60 encounters). In the presented figure, $\tau = 1$ and $\alpha = 0.5$, indicating that at least two successful signal 1/ TCR binding encounters with DCs have to occur in order for the signal to reach $\tau = 1$. Just by adding an additional requirement of another successful encounter in order for a T cell to be stimulated, lowered the probability of autoimmunity by 0.00768 fold (with serial encounter model/without serial encounter model=4.2e-05/5.5e-03). ($p_1 = p_2 = p$, $\tau = 1$, $alpha = 0.5$,, $d = 1$, $n_1 = 4000$, $n_2 = 60$)

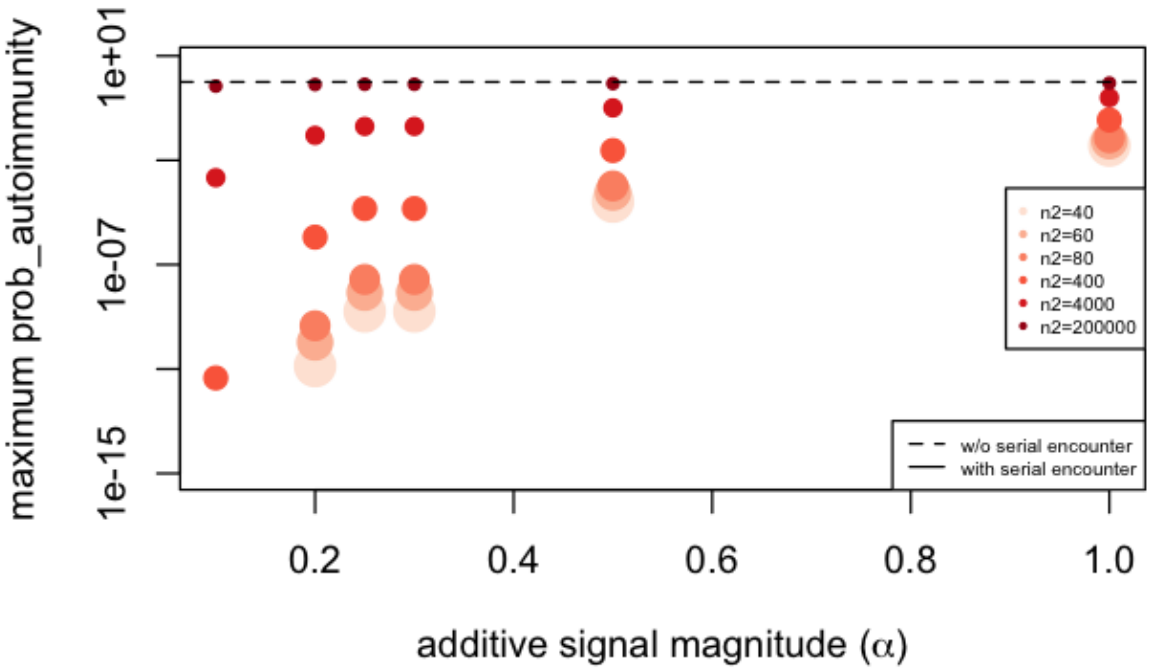


Figure 5: **Maximum probability of autoimmunity decreases with alpha but increases with n2.** The maximum probability of autoimmunity is the highest probability of autoimmunity value for a specific set of thymic vs. peripheral conditions over a range of different p values. The highest probability of autoimmunity in the simple model is when p=1e-3.6, corresponding to a probability of autoimmunity at 0.0055 (dashed horizontal line). As expected, if $\alpha = 1$, then the serial encounter model becomes the same as the simple model (no need for multiple successful encounter), thus in those cases where $\alpha = 1$, the maximum probabilities of autoimmunity under those conditions are the same as the simple model (top right dots). However, as α decreases, more successful encounters need to be achieved within the same 60 encounters considered during one lymph node priming series. Thus, it is harder for T cells to be activated under those conditions. As a result, the maximum probabilities of autoimmunity decrease as α decreases. Moreover, as n_2 increases, more encounters are being considered. When we evaluate $n_2 = 200,000$ encounters, which is an approximation of the total number of T-DC encounters made during a T cell's life time (half life of 1.5 years), the signals accumulate enough to reach τ, thus the mamimum probabilities of autoimmunity approaches 1. ($p_1 = p_2 = p$, $\tau = 1$, $\alpha = 0.5$, $d = 1$, $n_1 = 4000$, n_2 varies)

Chapter 4

Model 3- Serial Encounter Model with decay / decay model

As shown in Figure 5, if we relax the assumption that the integrated signal memory is cleared after each lymph node scanning, as the number of peripheral encounters increases (n_2), the probability of T cell activation approaches 1. If we consider more encounters, it is more probable that the signals will accumulate enough to reach the threshold for stimulation. Biologically, we know that these signals cannot sustain indefinitely. Through virtual lymph node stimulations, researchers have found that integrated signals during T cell priming for activation last for ≈2 days[29]. In other words, the integrated signals are stored in the memory space for a definite time interval, and this memory of integrated signals can decay.

4.1 Model construction

Here we introduce one other parameter, the decay rate, d. In our present model of a serial encounter model with decay, during T cell DC interactions, not only does the signal increase by $+a$ upon a successful encounter, but also decrease by $\times d$ when it is unsuccessful.

In our model, time is a discrete variable and we assume that one time interval is one T-DC interaction. Computationally, if the encounter is successful (determined by a

randomly generated probability compared to p), then the integrated signal increases by a ($+a$); if the encounter is unsuccessful, then the integrated signal decays by factor d ($\times d$). The decay factor d is a multiplicative fraction: a smaller d means that the decay is more rapid whereas a larger d is representative of a slower decay of the integrated signal (Table 3). When the overall integrated signal S_i reaches the threshold $\tau = 1$ after $i = n_2$ peripheral encounters, the T cell is considered activated. We then calculate the overall probability of stimulation after 100,000 simulations (Algorithm 2 execution code). Due to limitation of computational power, we chose small numbers of n_2 (40, 60) for the number of encounters in the periphery. Biologically, this is the approximate number of T-Dendritic cell encounters during one lymph node priming phase. This is similar to a biological local acute infection, and we assume that all DCs that the T cells encounter are activated. We make the same simplification for the conditions in the thymus as model 2, and evaluate how the decay model changes the probability of respective biological events.

Algorithm 2: Serial Encounter Model- decay model equation and execution

1

$$x_n \sim Bernoulli(p) \tag{4.1}$$

$$S_n = \begin{cases} d * S_{n-1}, & \text{if } x_n = 0 \\ S_{n-1} + a, & \text{if } x_n = 1 \end{cases} \tag{4.2}$$

$$P_s = P((\sum_{n=1}^{N} I(S_n \geq \tau)) \geq 1) \tag{4.3}$$

2 where

$$\tau = 1 \tag{4.4}$$

$$S_0 = 0 \tag{4.5}$$

$$n \in [1, N] \tag{4.6}$$

```
p<-function(N,a,d,p2) {
  pvals<-0
  for(i in 1:N){
    rand <- runif(1, 0, 1)
    if (rand<p2) {(pvals<-(a+pvals))}
    else {(pvals=pvals*d)}

    if(pvals>=1) break
    else i<-i+1

  }
  return(pvals)
}
```

Table 3: Model 3 Parameters

Abbreviation	Description	Values
p-MHC	peptide presented on MHC	
sp-MHC	self-peptide presented on MHC	
DC-self	self-peptides presented on *all* DC	
p	Probability of signaling (TCR binding) per encounter (Signal 1)	range [0,1]
$p_1 = p$	Probability of T cell clonal deletion per signal 1 encounter in the thymus	range [0,1]
$p_2 = p/k$	Probability of T cell activation per signal 1 encounter in the periphery	$k \geq 1$
k	Difference factorDifference in probability of apoptosis in thymus vs. actiation in the periphery	$k \geq 1$
α	magnitude of integrated signal per successful signaling event	$\alpha = seq(0, 1, 0.1)$
d	decay factor of integrated signal	range [0,1]
S_i	integrated signal value after ith encounter	range [0,1]
τ	threshold for stimulation (T cell activation occurs when $S_i \geq \tau$)	$\tau \geq 1$
n_1	number of DC encounters in thymus	4000
n_2	number of DC encounters in periphery	$n_2 \geq n_1$

4.2 Model results

The exact number of encounters in the periphery during one lymph node does not appear to make a difference as A,C,E panels are qualitatively similar to B, D, F panels. Thus, $n_2 = 60$ was used to generalize the conditions of a single lymph node series of serial encounter events (Figure 6).

During one lymph node encounter $n_2 \leq 60$, the model with decay has similar effects on the overall probability of autoimmunity given the same *alpha* level (Figure 1). But it is important to note that in the presented figure we showed the results of a scenario where the decay factor is only 0.7, thus the decay of the signal is slow.

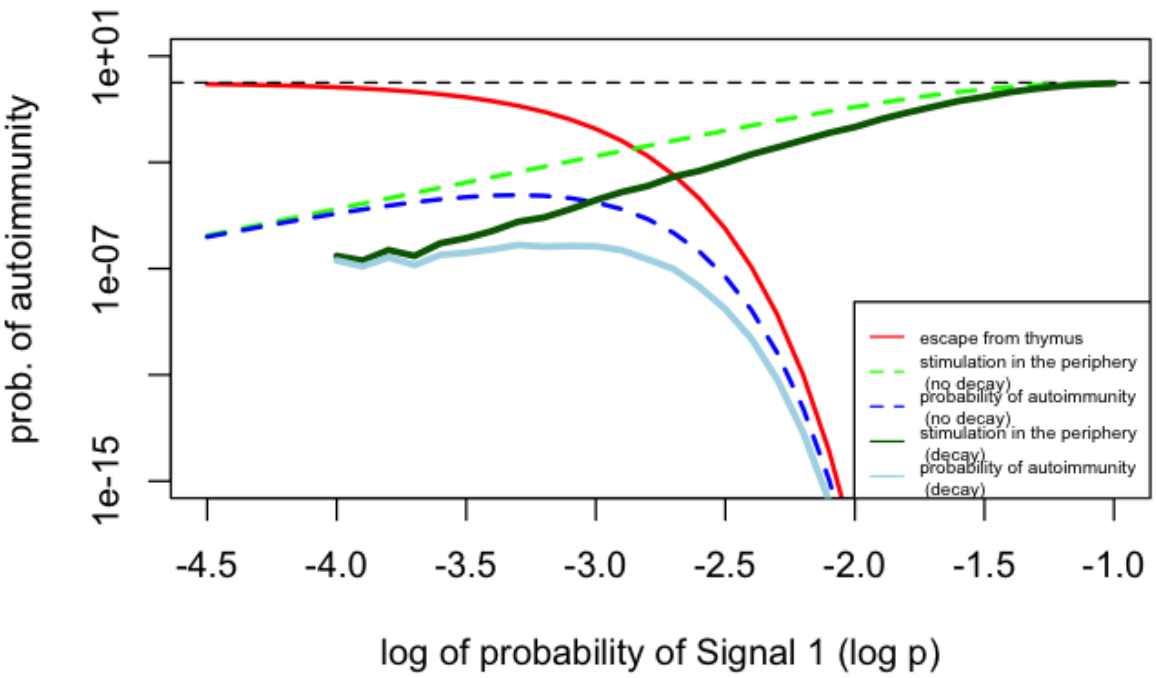


Figure 6: **Serial encounter model with decay lowers the probability of autoimmunity compared to a serial encounter model without decay during a short time interval.** With a serial encounter model, including a decay factor (d) for a short encounter window of 60 T-DC encounters in one local lymph node, the probability of autoimmunity (light blue solid line) is lower than the probability of autoimmunity without considering any decay (blue dashed line). The parameters to yield the current condition is similar to Figure 4 with $\alpha = 0.5$ but with the addition of a decay factor of $d = 0.9$. At least two successful encounters within the n_2 encounters of a lymph node have to be achieved in order to stimulate the T cell, and now the model includes a small decay factor for every unsuccessful encounter. ($p_1 = p_2 = p$,$\alpha = 0.5$, $d = 0.9$, $\tau = 1$ $n_1 = 4000$, $n_2 = 60$)

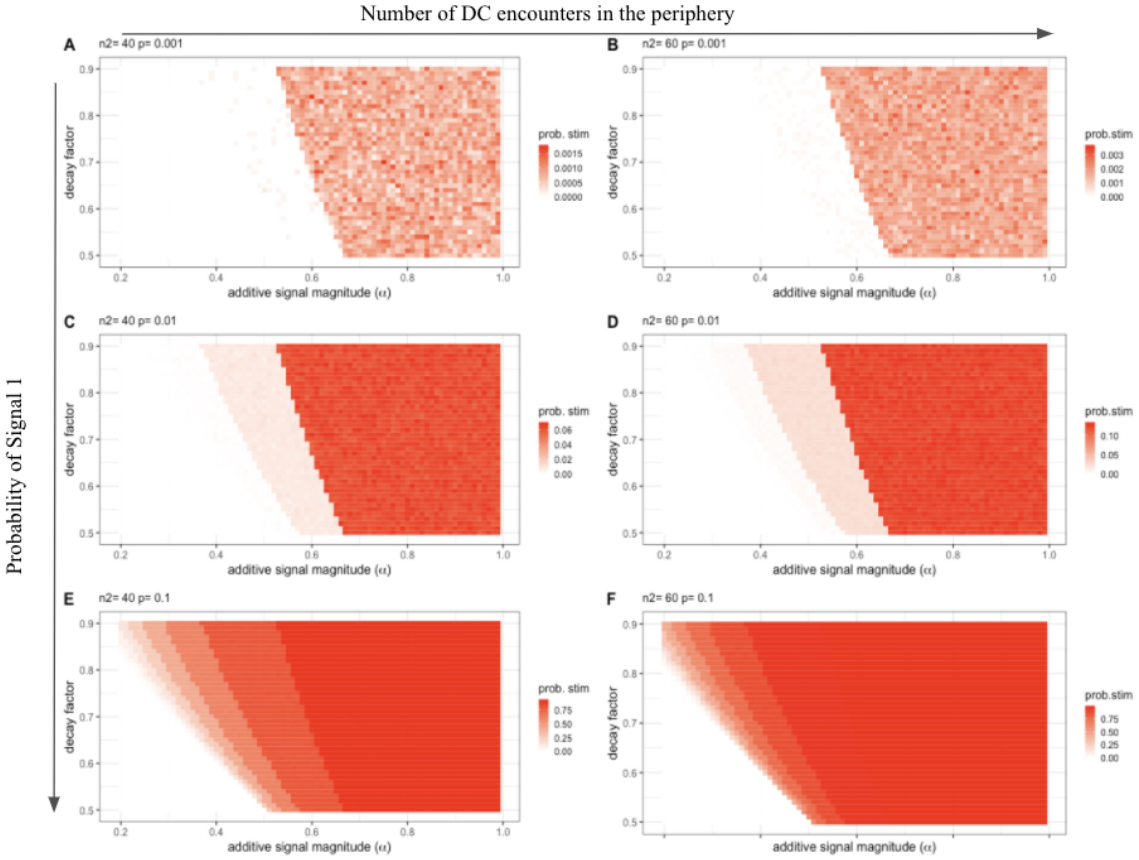


Figure 7: **The magnitude of the additive signal and decay factor of the signal impact the probability of peripheral stimulation of autoreactive T cells under a serial encounter model with decay.** As expected, T cells with higher avidity/affinity (p) to DC-self are more likely to get stimulated in the periphery as indicated by the change in scale and red pattern changes as p increases from A→ C→ E and from B→ D→ F. The exact number of encounters in the periphery during one lymph node does not appear to make a difference as A,C,E panels are qualitatively similar to B, D, F panels. As the additive signal α decreases, the probability of T cell stimulation is lowered. When α are d are both low (lower left hand corners), the probability of stimulation is magnitudes smaller (not zero but appears white because of its drastic difference in magnitude compared to the red regions). The decrease in decay factor d (more rapid decay) lowers the probability of stimulation even at the same level of α. Meanwhile, this decrease in d sharpens the threshold for stimulation: as shown in panels E and F, the horizontal distance from the white region to the dark red region is smaller when d is smaller. Overall, these heatmaps demonstrate the combined effect of changing α, d, p. and n_2. (α varies, d varies, $p_1 = p_2 = p$ varies, $\tau = 1$ $n_1 = 4000$, n_2 varies)

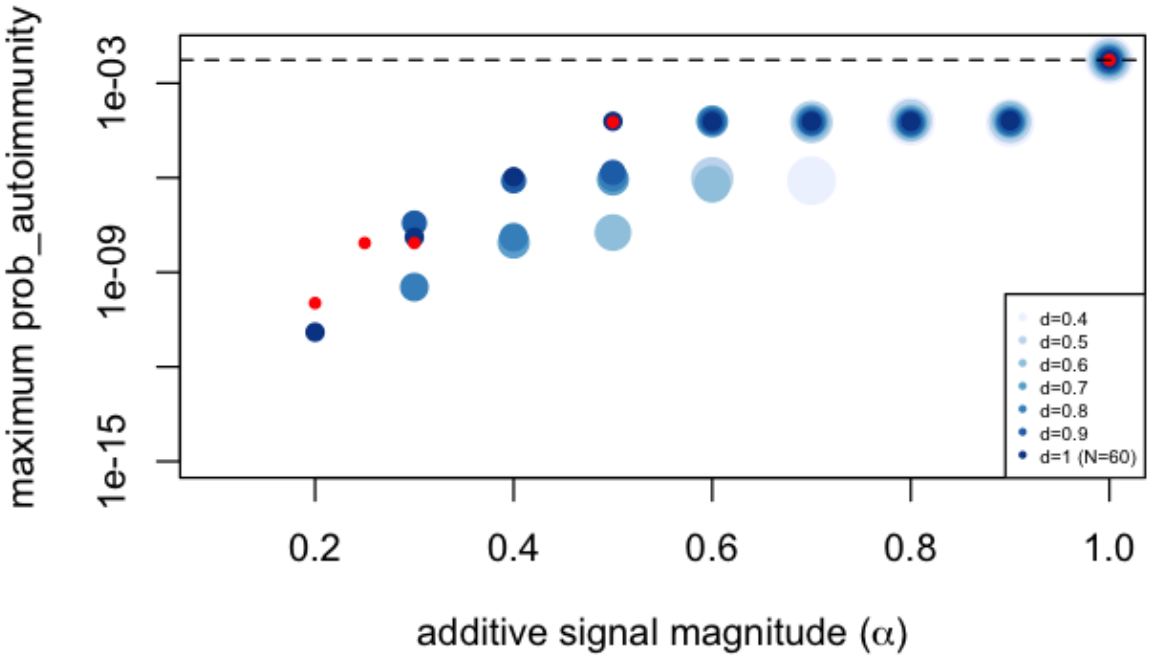


Figure 8: **Maximum probability of autoimmunity decreases as α and d decreases.** The maximum probability of autoimmunity is the highest probability of autoimmunity value for a specific set of thymic vs. peripheral conditions over a range of different p values. When only considering 60 encounters in one local lymph node, the highest probability of autoimmunity in the simple model is when p=1e-3.6, corresponding to a probability of autoimmunity at 0.0055 (dashed horizontal line). As expected, if $\alpha = 1$, then the serial encounter model becomes the same as the single-hit model (no need for multiple successful encounter), thus in those cases where $\alpha = 1$, the maximum probabilities of autoimmunity under those conditions are the same as the model 1 (top right dots). However, as α decreases, more successful encounters need to be achieved within the same 60 encounters considered during one lymph node priming series. As a result, the maximum probabilities of autoimmunity decrease as *alpha* decreases. Moreover, the decay factor also lowers the probability of stimulation in the periphery as the integrated signals are lost during intermittent signaling events. As d decreases, so does the maximum probability of autoimmunity (lighter color corresponds with a lower decay factor, meaning that the signal is lost faster). Compared to the red dots (maximum probabilities of autoimmunity at respective α levels under serial encounter model without decay / model 2), when d=1, the decay model yields similar results to model 2, as expected. There are some minute differences when $\alpha = 0.3$ due to the decay factor model being a computational model with randomness in its limited numbers of simulations. Nevertheless, the comparison between serial encounter model (model2) and decay model (model 3) with similar α and n_2 conditions demonstrate that the decay factor is important in decreasing the probability of autoimmunity over time even in a small time frame of just 60 encounters in one lymph node. ($p_1 = p_2 = p$, $\tau = 1$, α varies, d varies (blue), $d = 1$ (red), $n_1 = 4000$, $n_2 = 60$)

As the additive signal *alpha* increases, fewer successful encounters are required during the n_2 encounters to stimulate a T cell, thus it is easier for a T cell to get stimulated (color pattern gets darker as *alpha* increases). More rapid decay combined (smaller d) with a smaller additive signal (smaller α) results in a drastic decrease in the probability of autoimmunity (white regions in all panels with their respective scales) (Figure 7). The decrease in decay factor d (more rapid decay) makes it harder for T cells to be stimulated given the same α. As a result, the decay factor is very successful in also decreasing the probability of autoimmunity (Figure 8). Previously in Figure 5, the maximum probability of autoimmunity decreases as α decreases, but also increases to 1 as the number of DC encounters increases. Given the same number of DC encounters in the periphery $n_2 = 60$, having a decay factor in the model is effective in pulling down the probability of autoimmunity (blue dots) compared to the serial encounter model without decay (red dots). Thus, the addition of a decay factor in model 3 further prevents autoimmunity when we relax the assumption that the signal memory is cleared after each lymph node priming series. The decay factor allows this memory to be carried to other lymph nodes for a limited amount of time depending on d.

Interestingly, the level of avidity/affinity (logp) value corresponding to the maximum probability of autoimmunity increases as α decreases (Figure 9). This result suggests that the problematic T cells that will most likely cause autoimmunity are more reactive against the sp-MHC as peripheral T cell activation requires more successful encounters. This observation confers that the serial encounter model is in place as a fail-safe mechanism to prevent autoimmunity unless the cognate signal is very strong.

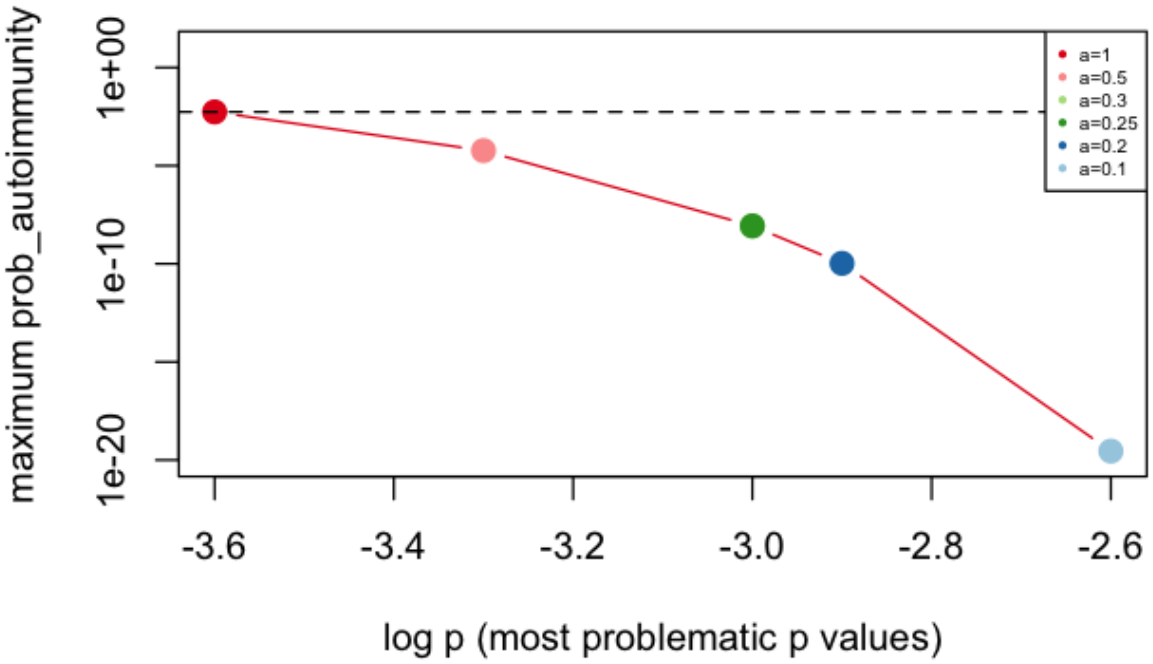


Figure 9: **The avidity/affinity of T cells corresponding to the maximum probability of autoimmunity increases when more successive encounters are required for T cell stimulation.** The maximum probability of autoimmunity is the highest probability of autoimmunity value for a specific set of thymic vs. peripheral conditions over a range of different p values. As we calculated before, the maximum probability of autoimmunity in the simple model (model 1) is when $p = 10^{-3.6}$. However, with the serial encounter model, the p value that corresponds with the maximum probability of autoimmunity increases. When the requirement for stimulation is stricter (α decreases), then it is harder for autoreactive T cells to be stimulated, thus the maximum probabilities of autoimmunity decrease, but the p values corresponding to the maximums increase. This suggests that the higher affinity/avidity T cells against DC are now becoming the problematic "intermediate" region. They are less problematic because the maximum probabilities of autoimmunity are lower, but the range of T cells that are problematic shifts towards the more reactive T cells. ($\tau = 1$, α varies, $d = 1$, $n_1 = 4000$, $n_2 = 60$)

Chapter 5

Discussion

Our results suggest that the complex pattern of T cell priming in secondary lymphoid organs may arise, at least in part, to prevent the stimulation of autoimmune T cells responses to DC-self. We have demonstrated that multiple encounters, in addition to central tolerance, both contribute to the generation of immune tolerance against DC-self specific T-cells.

5.1 Our Models

In the single hit model, we first assumed that the T-DC interactions are the same both in the thymus and in the periphery. We then developed a set of computational models to quantitatively assess how various peripheral mechanisms such as T cell activation rules of multiple serial encounters contribute to the overall calculations of the probability of autoimmunity. Our results demonstrated that the probability of autoimmunity is the highest for a T cells with affinities/avidities at the subthreshold level for thymic selection (Figure 1), as they barely escape thymic selection and are more likely to be stimulated in the periphery due to its affinity/avidity for DC-self. The serial encounter model is effective in lowering the probabilities of peripheral stimulation and autoimmunity (Figure 4) but a decay factor is necessary in regulating the longevity of the additive integrated signals (Figure 8). Moreover,

the results suggested that the level of strictness of the serial encounter model, indicated by the α levels is an important determining factor in regulating the probability of autoimmunity (Figure 5, Figure 7). These α levels are chosen arbitrarily to understand the ratio of signal vs. threshold level. Here our threshold for stimulation is $\tau = 1$, and the α levels are easier to interpret. Biologically, these thresholds may be subject to change depending on the environmental conditions.

From our models, we concluded that DC-self reactive T cells cannot be effectively eliminated just by thymic selection alone, and the differences in T cell dynamics in the periphery vs. thymus have to be closely assessed. If we assume that the conditions of the thymus and the periphery are the same for DC-self specific T cells, as suggested in Matzinger's hypothesis, then we observe a high probability of autoimmunity against dendritic cells. However, the serial encounter model proposed in our models does not complete the story either, as explained in Section 3.2 and 4.2.

5.2 Efficiency of The System

From the set of mathematical models above, we have demonstrated how various peripheral mechanisms of T cell activation, in addition to central tolerance, prevent autoreactive DC-self specific T-cells from being stimulated and causing autoimmunity. However, these mechanisms that lower the probability of peripheral stimulation and in turn lower the probability of autoimmunity also lowers the probability of immune activation upon an infection. The affinity/avidity values of T cells we used to construct our model represents the probability of a T cell encountering a successful Signal 1 against a specific antigen, in which we defined its specificity to be for a DC-self peptide. Meanwhile, other T cells with various distributions of p for foreign peptides are also subjected to the same mechanisms of central tolerance, and serial encounter requirements for peripheral stimulation. The behavior of T cell stimulation in our models is universal for T cells regardless of its cognate antigen. T

cells specific for ovalbumin may escape central tolerance due to its low avidity and affinity for self-peptides, but they are less likely to be stimulated upon encountering ovalbumin as multiple successful encounters are required to stimulate a T cell. This fail-safe mechanism for preventing stimulation of T cells in the periphery may in fact hinder the immune system's ability to efficiently respond to an infection.

By making it harder for T cells with specific affinity/avidities (p) to get stimulated in the periphery with stricter activation requirements (lower α), there exists a tradeoff between decreasing the probability of autoimmunity and decreasing the immune system's sensitivity against foreign antigens. T cells are cross-reactive in nature. A specific T cell may have a low affinity against DC-self but it may have a high specificity against a foriegn antigen[3]. In fact, the diversity of the immune repertoire is a theoretical puzzle that has gained much attention, and some have proposed that the diversity of the T cell receptor is determined by the number of self-peptides it has to avoid reactivity against[30]. This leads us to the crucial issue we face in terms of contextualizing our results: We do not know the empirical distribution of self-reactive T cells against DC-self, nor do we know how cross-reactive the escaped T cells are against a foreign antigen. Moreover, in order to understand the tradeoff between autoimmunity and the specificity of the immune-system, the same problem is present. We do not quantify this tradeoff because of the limitation in quantifying the distribution of T cell reactivity. We cannot conclude what level of probability of autoimmunity is sufficient for the immune system because of the exact reasons mentioned above. We do not know how many DC-self reactive T cells exist in our immune systems, and we do not know their distributions of affinities. Needless to say, we can make general inferences about what ranges of affinities are problematic and how different T cell activation requirements contribute to the system's behavior.

However, by looking at the fold change difference (probability of stimulation in model2/ probability of stimulation in model 1), we see that the effect of serial encounter makes the most difference when the affinity/avidity of the T cell (p) is low against its

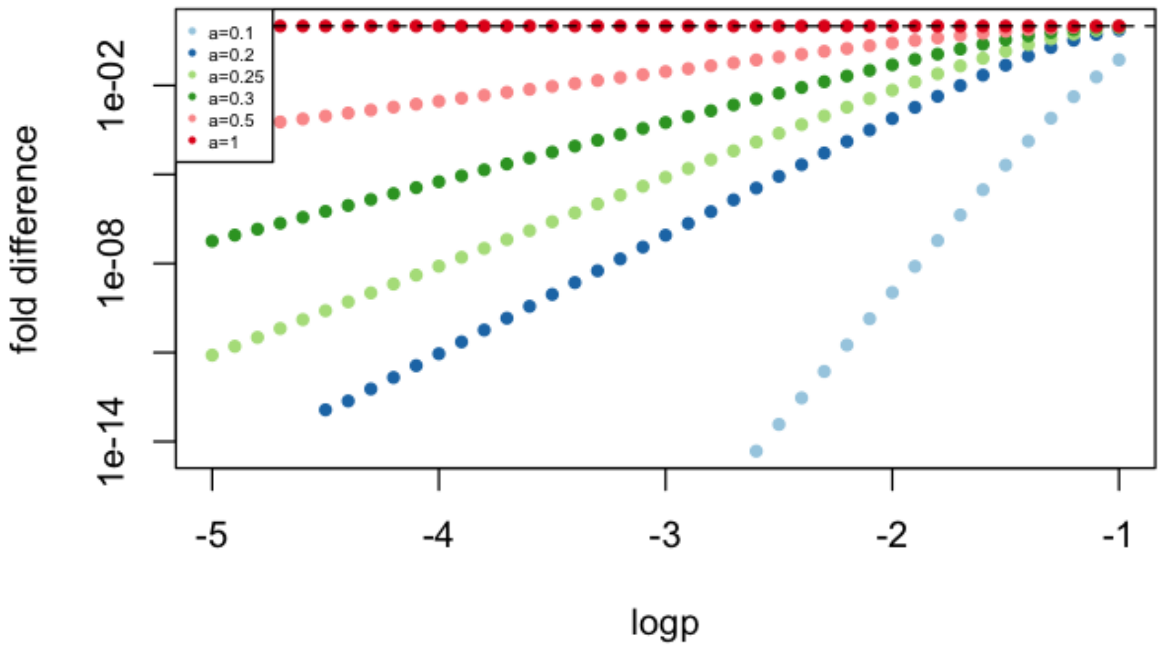


Figure 10: **Comparison of serial encounter model (model 2) vs. no serial encounter model (model 1).** The probability of autoimmunity in the serial encounter model over the probability of autoimmunity in model 1 (single-hit model) is calculated as the fold difference. When $\alpha = 1$, the serial encounter model behaves similarly to the single-hit model, thus the fold difference is 1 (black dashed line). The fold difference decreases as the avidity/affinity of the TCR (logp/probability of signal 1) increases, indicating that the effect of serial encounter is more prominent in lower ranges of logp. With a high logp, the serial encounter model does not decrease the probability of autoimmunity drastically. Moreover, different levels of α measures the degree of effect of the serial encounter model. Under a low α, the effect of the serial encounter model at lower logp is more prominent.

cognate antigen (Figure 10). For balancing efficiency of the immune system, we see that if a T cell has a high affinity/avidity (p) for a specific antigen, then the serial encounter model does not affect it drastically (a=1 vs. a=0.3). Moreover, if a T cell has a high p for an antigen, it will be deleted in the thymus if that antigen is a self-antigen (Figure 1). Thus, for T cells specific for foreign antigens, its specificity renders a high p, and serial encounter will not impair the efficiency of the immune system's response against a foreign antigen. If the T cell has a low p for an antigen, however, then the serial encounter model is a fail-safe in ensuring that the T cell is responding to the correct antigen under the correct stimulations.

5.3 Biochemical Relevance

Given the limitations of contextualizing the results of our models, our results still elucidate multiple biological realities and show how quantitatively different mechanisms come together to lower the probability of autoimmunity.

Biochemically, experimental results have supported the notion that transient, accumulated T cell activation signals contribute to T cell activation. Virtual lymph node simulations that are very similar to our current constructs have demonstrated that an additive signal with a memory of 1-2 days greatly mimics experimental results[29]. Molecularly, experimental evidence showed that transient intermittent TCR signals correlated with active transcription of tolerance genes but not effector genes, while continuous TCR triggering is required for the expression of effector genes[27]. By looking at the shuttling and transcriptional regulation behaviors of NFAT, a potential molecular mechanism was proposed to support the serial encounter model we simulated[27]. Moreover, experimental evidence tracking calcium signaling during T cell development indicated that there are specific quantitative and temporal requirements that determine a T cell's fate after TCR signaling (calcium),. Intermittent signaling and accumulation of TCR signaling events have been important in allowing for the proper intracellular secondary messengers and responses to carry on upon environmental simulations[31,28].

Signals that indicate thymic environment, peripheral environment, infection environment etc. all contribute to how T cell receptor binding to MHC presented peptides can result in various outputs and how these evolved mechanisms and pathways provide overall immune protection while preventing autoimmunity. We have seen various fail-safe mechanisms in place to ensure the immune system's proper response: serial encounters with DCs required to activate T cells is a redundancy in the immune system, which acts as a fail-safe to ensure that T cells are responding properly to its stimulus[32].

Another important fail-safe mechanism of the immune system in regulation T cell activation, that we have implicitly addressed in our models, is the fact that T cell activation

requires 2 signals, both TCR binding and costimulatory binding. We have assumed in our models that the all DCs that T cells encounter are activated, which is not exactly representative of biological realities. Under an infection, ≈50% DCs in the lymph nodes are activated[33], and this number is even lower when there is no present infection. During our simulations, we are assuming and replicating biologically a local acute infection where all DCs in the lymph node are activated, and we recognize that this assumption has to be re-evaluated in future models in order to properly assess T cell fates during its lifetime. In future models, we wish to incorporate more complex peripheral tolerance mechanisms such as anergy, which is partly dependent on the level of DC activation[34]. We will then assess different n_2 values to address different infection scenarios. For example, a local acute infection will use parameters similar to our current models, but a chronic infection will warrant much higher n_2 values. The steady state condition of a T cell without any present infections also has to be evaluated in combination of anergic phenotypes and behaviors.

One other biological relevance of our model parameters and model insights is the p value. In our current construct, p takes on a value between 0-1 and the distribution of p among the T cell reperiotre is unknown. Each T cell has a specific p value against a specific antigen. In this case, we are assessing the specific p value a T cell has against DC-self. Nevertheless, there is a biological significance to this p even though it incorporates many factors such as affinity and avidity together. Experimentalists can quantify p by measuring the duration of T-DC contacts to yield the TCR triggering rate, and calculate the avidity of cognate antigens in a local environment by assessing antigen presenting cells[35,17]. By studying individual TCR dynamics with a given peptide-MHC pulsed environment, some inferences and estimations can be made on what p looks like in an experimental setting, which can further inform experimentalists on the subsets of T cells that may be problematic for autoimmunity.

Meanwhile, another biological condition to consider when analyzing autoimmunity against DC-self, is that dendritic cells are more resistant to killing. Our model evaluates the

probability of T cells attacking DCs and causing autoimmunity. But DCs are important in immune functions and research has shown that mature DCs express serine protease inhibitors that inhibit granzyme B and thereby block cytotoxic killing[36]. This is also an important factor to consider when interpreting the numbers of probabilities of autoimmunity from our models. Our model takes on the perspective of the self-reactive cytotoxic T cell, rather than the perspective of the DCs.

5.4 Peripherial Tolerance Mechanisms

Peripheral tolerance mechanisms can be divided into two classes: T cell intrinsic mechanisms and T cell extrinsic mechanisms[18]. Our present model goes as far as looking at how T cell activation requirements in the periphery changes the probability of autoimmunity, and leaves room for peripheral tolerance mechanisms to come in. Thus, our current quantitative estimates are just upper bounds to the level of autoimmunity without considering the peripheral tolerance mechanisms that are crucial for T cell regulation and response.

Future work in addition to our modeling work can be extended to experimental explorations of anergy. Specifically, experimental results can help further elucidate between the different hypotheses of anergy, whether anergic T cells change their threshold for stimulation, change their magnitude of response to stimuli, or both. Moreover, computational aspects of this work can be extended to incorporate other peripheral tolerance mechanisms such as the role of regulatory T cells in the generation of biological robustness and preventing T cell autoimmunity. Interestingly, the autoimmune regulator randomly expresses peripheral tissue antigens on medullary thymic epithelial cells, and these are thought to play a role in both clonal deletion and the generation of regulatory T cells[37]. This peripheral tolerance mechanism will be an interesting complex puzzle to tackle in future work that builds off of the probabilistic models established here.

5.5 Beyond DC-self

We limited the analysis of probabilities of autoimmunity of T cells specific to DC-self in our present models because of the reasons listed earlier. However, the puzzle of how different mechanisms combine to contribute to the overall formation of immune tolerance is well beyond just DC-self specific T cells. In order to extend our models to other peripheral tissue antigen specific T cells, we will have to explore and incorporate AIRE regulated peripheral tissue antigen expression in medullary thymic epithelial cells and the other peripheral tolerance mechanisms.

Overall, this research has attempted to use probabilistic and computational models to simulate T cell interactions with dendritic cells through the development and initial peripheral conditions of a T cell. The models elucidated that addressing the differences between thymic and peripheral T-DC interaction is important in understanding how immune tolerance is achieved quantitatively. This research will contribute to broader understanding of fundamental questions regarding cellular decisions of T cells upon different stimulation conditions and the formation of immune tolerance.

Appendix A

Müller & Bonhoeffer

Müller and Bonhoeffer published an opinion in Trends in Immunology in 2003[9]. A lot of the work in this paper is based on their quantitative estimates. We used similar n_1 values as them to quantify the number of DC encounters in the thymus. This number was based on the calculation that:

Negative selection takes place in the thymic medulla of mouse models 5-14 days. 14 days ($2x10^14$ minutes) and the time needed for a thymocyte to scan one DC has been estimated to be 6-12 minutes. Assuming a 5 minute scanning time, the upper bound for the number of DC scanned in 14 days is $2x10^14/5 = 4000$.

Müller and Bonhoeffer quantify the limitations of negative selection by assuming that DCs present peripheral tissue antigens to T cells in the thymus in a uniform distribution. And it makes calculations and estimations of the potential presentation of a dendritic cell. And the process of selection is determined based on how many times the T cell encounters the same antigen on the same DC. For example, if the threshold for negative selection was 10 identical sp-MHC complexes (x), and a specific dendritic cell was capable of presenting a total of 100 sp-MHCs (N), Müller and Bonhoeffer claims that the dendritic cell can present 100/10=10 (C_p) different distinct peripheral tissue antigens. Then by comparing the ratio of a DC's total presentation capability with the total number of peripheral tissue antigens (C_t),

this ratio yields the probability that an sp-MHC is presented by a DC above the threshold for negative selection (p). The probability of thymic escape is then calculated by $(1-p)^t$ where $t = 4000$ (Table 4).

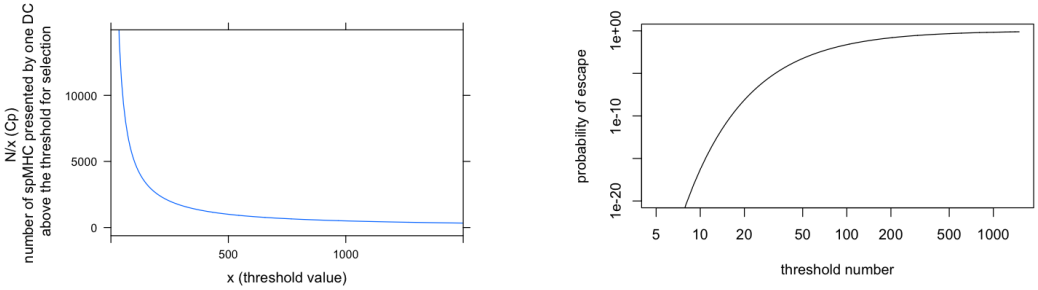
Table 4: Parameters for Muller & Bonhoeffer

Abbreviation	Description	Values
p-MHC	peptide presented on MHC	
sp-MHC	self-peptide presented on MHC	
DC-self	self-peptides presented on *all* DC	
x	threshold value for number of cognate spMHC necessary to mediate negative selection	range [0,1500]
N	total number of spMHC on the surface of DC (for mTEC $N = 5e4$)	$N = 5 * 10^5$
C_p	number of distinct complexes presented by one DC	$C_p = N/x$
C_t	total number of presentable spMHCs	$C_t = 3e7 * 0.03 * 6 = 5.4e6$
p	probability that an spMHC is presented by a DC above the threshold for negative selection	$p = C_p/C_t$
t	number of DCs scanned during negative selection (considers time during N.S)	4000 $time = 14days/5minutes$
P_E	probability of escpaing the thymus	range [0,1] $P_E = (1-p)^t$

Based on this construction, as the threshold value increases, the presentation capability of a DC decreases and the probability of thymic escape increases (Figure S1). Müller and Bonhoeffer claimed that with a high presentation threshold required for DCs, it is very likely that self-reactive T cells will escape the thymus.

However, there are many problems with their framework. One of the most critical flaws of this construct is the fact that DCs do not present all peripheral tissue antigens in the thymus, and they do so in low concentrations that are not uniform for different antigens. Given this critical flaw, we could not accept the claims that Müller and Bonhoeffer made. And from the perspective of a DC presentation, this is not a comprehensive way to understand negative selection.

Müller and Bonhoeffer's work, nevertheless, is one of the first theoretical papers aiming to quantify the efficiency of negative selection and its focus on DCs did foster interesting conversations in the field. They inferred that T cells specific for DC-self were possible to be deleted given their construct as they lowered N to a small subset of DC-specific self peptides. However, their evaluation of Matzinger's hypothesis is limited. We wish to improve on their work and evaluate Matzinger's hypothesis through the lens of a DC-self reactive T cell.



(a) label 1 (b) label 2

Figure S1: A.The number of spMHC presented by one DC above the threshold for selection decreases as the threshold value increases. B: The probability of escape from negative selection increases as the number of cognate spMHC necessary to mediate negative selection (threshold) increases

Appendix B

Chan et al.,

Cliburn Chan et al. published in the Journal of Computational and Applied Mathematics in 2005 a mathematical model investigating the impact of anergy as well as multiple T cell-APC encounters on the probability of autoimmunity[17].

We adopted a lot of the constructs from the Chan paper in our presented models. Conceptually, Chan et al. analyzed the autoimmunity and efficiency of negative selection from the perspective of a T cell and its ability to be stimulated. Interestingly, Chan et al. quantified the strength of signal 1 using a sigmoid function.

$$f(s) = \frac{s^m}{(s^m + \theta_1^m)}$$

activation probabtility

Signal 1 (s) is the product of the number of cognate peptide-MHCs on the cell surface and the triggering rate, which is calculated from the process of serial ligation and kinetic proofreading.

They changed the sigmoid function and different parameters to explore the roles and interactions of different stimulation thresholds (θ), and anergy. Chan et al. showed that stochastic activation of T cells in the context of different activation thresholds for T cells and defined anergy as a state of T cell response when the threshold for activation is not met,

but some signaling is present:

$$f(s)_{anergy} = \frac{s^m}{(s^m + \theta_2^m)} - \frac{s^m}{(s^m + \theta_3^m)}$$

anergy is a result of a weak s between $\theta_2$and θ_3, which is 1/20 and 1/10 if θ_1

From these calculations, Chan suggested that when the signal 1 is strong, then T cells are more likely to be stimulated, whereas when the signal is low, T cells are more likely to be anergic (Figure S2).

Additionally, Chan et al. quantified the probability of autoimmunity as a product of the probability of thymic escape and probability of peripheral stimulation:

$$\text{prob. autoimmunity} = P_A = P_E \times P_S = \underbrace{(1-p_1)^{n_1}}_{\text{prob. of no stim in thymus}} \times \underbrace{1-(1-p_2)^{n_2}}_{\text{prob. of stim in periphery}}$$

From their calculations, they demonstrated that multiple encounters in the periphery lowers the threshold of T cell activation and that anergy is important in lowering the probability of autoimmunity.

They also attempted to examine the trade-off of specificity of immune responses for sensitivity to foriegn antigens by assuming that $< 1\%$ total MHCs bear foireign antigens,a dn 1% of naive T cells chosen at random will recognize the foreign antigen presented by the pathogen loaded DC. However, these assumptions and arbitrary numbers they used to understand the efficiency of the immune system is largely arbitrary with unpredictable biological truth.

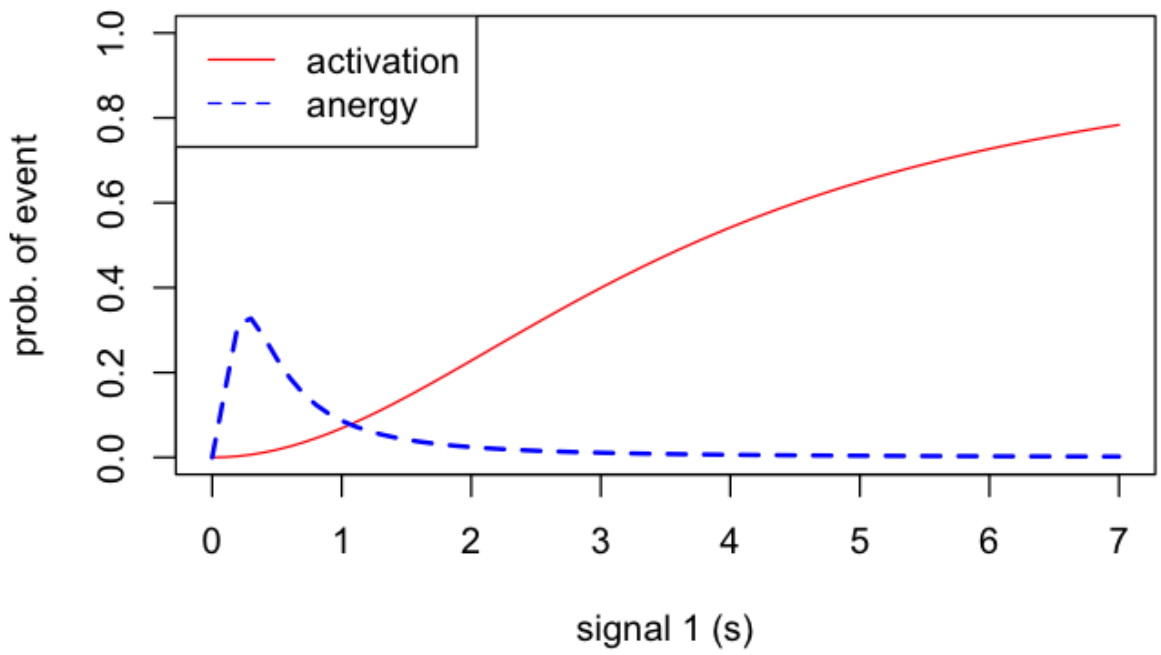


Figure S2: The probability of T cell anergy/activation is a function of signal 1 (s) received during a single interaction with an APC. Deletion in the thymus is covered by a similar function as for activation. The probability that a cell does nothing during a peripheral encounter is 1-P(activation)-P(anergy). Parameters: $\theta_1 = 10e^{-}1, \theta_2 = 0.05\theta_1, \theta_3 = 0.1\theta_1$

Appendix C

Affect of changing n_2 and k in model 1

If we plot P_A as a function of p we see P_A can be considerable.

$$\text{prob. autoimmunity} = P_A = P_E \times P_S = \underbrace{(1-p_1)^{n_1}}_{\text{prob. of no stim in thymus}} \times \underbrace{1-(1-p_2)^{n_2}}_{\text{1-prob. of no stim in periphery}}$$

An upper bound to the equation can be written as:

$$P_A = \underbrace{(1-p_1)^{n_1}}_{\text{prob. of no stim in thymus}} \times \underbrace{n_2 p_2}_{\text{1 - prob. of no stim in periphery}})$$

The derivative of this function is:

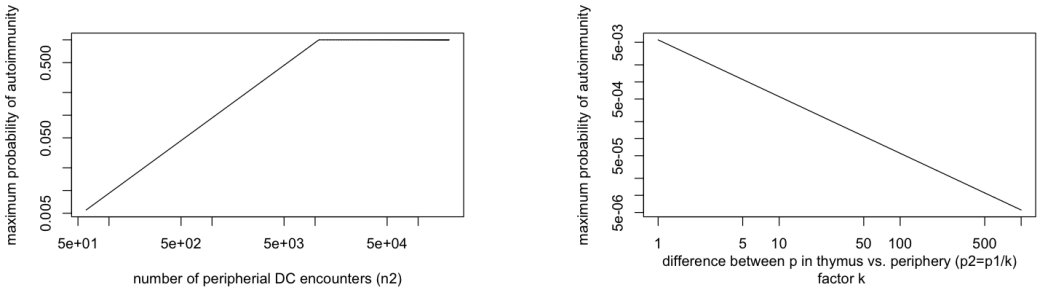
$$\frac{d}{dp}\left[n_2\left(\frac{p}{k}\right)(1-p)^{n_1}\right] = 0$$

or

$$\frac{n_2}{k}(1-p)^{n_1} = n_2\left(\frac{p}{k}\right)n_1(1-p)^{n_1-1}$$

which gives

$$(1-p) = pn_1$$



(a) label 1 (b) label 2

Figure S3: A. The maximum probability of autoimmunity is directly positively proportional to the number of peripheral DC encounters (n_2). B. The maximum probability of autoimmunity is inversly/negatively proportional to factor k (k)

or

$$p = \frac{1}{n_1 + 1} \approx \frac{1}{n_1}$$

so

$$\max(P_A) = n_2 \left(\frac{1}{n_1 k}\right) \left(1 - \frac{1}{n_1}\right)^{n_1}$$

we know probabilities are less than 1 so

$$P_A = \min\left(1, n_2 p_2 (1 - p_1)^{n_1}\right)$$

$$P_{maxA} = \min\left(1, n_2 \left(\frac{1}{n_1 k}\right) \left(1 - \frac{1}{n_1}\right)^{n_1}\right)$$

From this equation, we see that we can plot $\max(P_A)$per cell vs m and k and note that it increases linearly with lifespan n_2 and decreases linearly with k.

Appendix D

Q & A

(i) Why is N2 only 60?

a) We recognize that during the duration a T cell spends in the periphery, T cells make >60 encounters with DCs. However, without a decay model, as n2→ inf probability of autoimmunity → 1. And when there is a signal decay, since our current computation takes a lot time to run, we did not explicitly evaluate greater numbers of n2 until we find an analytical solution to the decay model.

b) In our models, we assumed that there is a local acute infection, and all DCs that the T cells encounter are activated. We also assumed time is a constant variable in between the different encounters, thus every encounter is one unit of time. This assumption may be re-assessed in future studies considering that encounter time differs kinetically based on DC MHC complex density, density of DCs, and antigen load. Here we only considered a simple scenario to indicate the affect of serial encounters.

c) In the current framework, ignoring special features of RTEs, these potentially self-reactive T cells escape from the thymus and we are assessing its probability of autoimmunity during its priming phase in one lymph node (indirectly, we are clearing its memory compartment every time it migrates to another lymph node).

Mempel et al., have showed that the priming phase of T cells is 4-8 hours at a rate of 6min/contact with DCs. And if the T cells do not get fully stimulated during its priming phase in the local lymph node, it migrates to another one. These are the premises of the n_2 values we chose.

(ii) Why only DC self?

a) In order to have a strong foundation and be the most realistic with our estimations, we wanted to look at central tolerance in a simple form similar to Muller & Bonehoeffer– Looking at peptides presented by DCs. We then encountered the idea that DCs also present their self-peptides in the thymus.

b) Dr. Polly Matzinger has hypothesized that because DCs are so important in the role of immune functioning, and their similar expressions in the thymus, periphery. It is possible and integral to delete DC-self reactive T cells.

(iii) What about peripherial tolerance mechanisms

a) We recognize that the immune system and T cells are not isolated, peripheral tolerance mechanisms are important to consider, and this estimation is an upper bound.

b) Peripheral tolerance mechanisms have their own quantitative impacts on preventing autoimmunity

c) Future works will be dedicated to incorporating anergy into our model as it seems to be the most relevant T-cell intrinsic peripheral tolerance mechanism at play. We hope to answer through quantitative estimations the question, why keep anergic T cells around.

d) Future works after incorporating anergy will then be to extend to T-cell extrinsic peripheral tolerance mechanisms such as tolerant DC cells, and the roles of regulatory T cells.

(iv) What does probability of autoimmunity mean contextually?

a) The numbers are hard to interpret, what does 1e-5 probability of autoimmunity mean? In our context, this means that for a T cell with (p) probability of correct peptide MHC binding/signal1 (including affinity and avidity), it has 1e-5 probability of causing autoimmunity in our simple framework ignoring peripheral tolerance.

b) T cells are inherently cross-reactive, thus we do not know the distribution of all DC-self reactive T cells. We do not know how many T cells fall exactly under each (p) value range. We recognize this as a limitation to our current model, and do not yet have the best way to estimate this empirically.

c) Though the main goal of this project is to quantitatively understand to what magnitude do all the discussed mechanisms such as serial encounter impact the system and why it may be required as a fail safe mechanism of the immune system.

(v) Efficiency of the system?

a) Since the serial encounter model not only lowered the probability of autoimmunity, but also decreased the probability of a T cell being stimulated in the periphery, it is important to note that the serial encounter model is a double-edged sword.

b) Decreasing the probability of T cell stimulation decreases the probability of autoimmunity if the T cell is self-reactive, but also decreases the efficiency of detection and response to an infection if the T cell is reactive against a foreign peptide. The trade-off between the specificity of the immune system (correctly identifying an antigen) vs. the sensitivity (correctly responding to an infection) is hard to measure because, again, T cells are cross-reactive. And being self-reactive vs. reacting against a foreign peptide is not a binary measurement.

c) We do our best to acknowledge this trade-off and evaluate it verbally.

(vi) What do we get out of the current model? What is new?

a) We evaluated the Matzinger's hypothesis and have demonstrated that even for DC-self reactive T cells, central tolerance is not complete.

b) We quantitatively demonstrated the effect of an signal integration model and its role in impacting stimulation and regulation of the immune system.

Bibliography

[1] Craig H. Bassing, Wojciech Swat, and Frederick W. Alt. The mechanism and regulation of chromosomal V(D)J recombination. *Cell*, 109 Suppl:S45–55, April 2002. ISSN 0092-8674. doi: 10.1016/s0092-8674(02)00675-x.

[2] Yueh-hsiu Chien, Nicholas R. J. Gascoigne, Joshua Kavaler, Nadine E. Lee, and Mark M. Davis. Somatic recombination in a murine T-cell receptor gene. *Nature*, 309(5966):322–326, May 1984. ISSN 1476-4687. doi: 10.1038/309322a0. URL `https://www.nature.com/articles/309322a0`. Number: 5966 Publisher: Nature Publishing Group.

[3] Andrew K. Sewell. Why must T cells be cross-reactive? *Nat Rev Immunol*, 12(9): 669–677, 2012. ISSN 1474-1733. doi: 10.1038/nri3279. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7097784/`.

[4] Veronika I. Zarnitsyna, Brian D. Evavold, Louis N. Schoettle, Joseph N. Blattman, and Rustom Antia. Estimating the Diversity, Completeness, and Cross-Reactivity of the T Cell Repertoire. *Front Immunol*, 4, December 2013. ISSN 1664-3224. doi: 10.3389/fimmu.2013.00485. URL `https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3872652/`.

[5] F. M. Burnet. A modification of Jerne's theory of antibody production using the concept of clonal selection. *CA Cancer J Clin*, 26(2):119–121, April 1976. ISSN 0007-9235. doi: 10.3322/canjclin.26.2.119.

[6] H. von Boehmer, H. S. Teh, and P. Kisielow. The thymus selects the useful, neglects the useless and destroys the harmful. *Immunol Today*, 10(2):57–61, February 1989. ISSN 0167-5699. doi: 10.1016/0167-5699(89)90307-1.

[7] Andrew Yates. Theories and Quantification of Thymic Selection. *Front. Immunol.*, 5, 2014. ISSN 1664-3224. doi: 10.3389/fimmu.2014.00013. URL `https://www.frontiersin.org/articles/10.3389/fimmu.2014.00013/full`. Publisher: Frontiers.

[8] P Matzinger. Tolerance, Danger, and the Extended Family. *Annu. Rev. Immunol.*, 12(1):991–1045, April 1994. ISSN 0732-0582. doi: 10.1146/annurev.iy.12.040194.005015. URL `https://www.annualreviews.org/doi/10.1146/annurev.iy.12.040194.005015`. Publisher: Annual Reviews.

[9] Viktor Müller and Sebastian Bonhoeffer. Quantitative constraints on the scope of negative selection. *Trends in Immunology*, 24(3):132–135, March 2003. ISSN 1471-4906. doi: 10.1016/S1471-4906(03)00028-0. URL `http://www.sciencedirect.com/science/article/pii/S1471490603000280`.

[10] Diane Mathis and Christophe Benoist. A decade of AIRE. *Nature Reviews Immunology*, 7(8):645–650, August 2007. ISSN 1474-1741. doi: 10.1038/nri2136. URL `https://www.nature.com/articles/nri2136`. Number: 8 Publisher: Nature Publishing Group.

[11] Dietmar Zehn and Michael J. Bevan. T Cells with Low Avidity for a Tissue-Restricted Antigen Routinely Evade Central and Peripheral Tolerance and Cause Autoimmunity. *Immunity*, 25(2):261–270, August 2006. ISSN 1074-7613. doi: 10.1016/j.immuni.2006.06.009. URL `https://www.cell.com/immunity/abstract/S1074-7613(06)00342-6`. Publisher: Elsevier.

[12] Sarah Enouz, Lucie Carrié, Doron Merkler, Michael J. Bevan, and Dietmar Zehn. Autoreactive T cells bypass negative selection and respond to self-antigen stimulation during infection. *Journal of Experimental Medicine*, 209(10):1769–1779, September 2012.

ISSN 0022-1007. doi: 10.1084/jem.20120905. URL https://doi.org/10.1084/jem.20120905.

[13] Mark A. Daniels, Emma Teixeiro, Jason Gill, Barbara Hausmann, Dominique Roubaty, Kaisa Holmberg, Guy Werlen, Georg A. Holländer, Nicholas R. J. Gascoigne, and Ed Palmer. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature*, 444(7120):724–729, December 2006. ISSN 1476-4687. doi: 10.1038/nature05269. URL https://www.nature.com/articles/nature05269. Number: 7120 Publisher: Nature Publishing Group.

[14] C. Bouneaud, P. Kourilsky, and P. Bousso. Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. *Immunity*, 13(6):829–840, December 2000. ISSN 1074-7613. doi: 10.1016/s1074-7613(00)00080-7.

[15] D. Hawiger, K. Inaba, Y. Dorsett, M. Guo, K. Mahnke, M. Rivera, J. V. Ravetch, R. M. Steinman, and M. C. Nussenzweig. Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions in vivo. *J Exp Med*, 194(6):769–779, September 2001. ISSN 0022-1007. doi: 10.1084/jem.194.6.769.

[16] Marc K Jenkins and Ronald H Schwartz. Antigen presentation by chemically modified splenocytes induces antigen- specific T cell unresponsiveness in vitro and in vivo. *J Exp Med*, 165(2):302–319, February 1987. ISSN 0022-1007. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2188516/.

[17] Cliburn Chan, Jaroslav Stark, and Andrew J. T. George. The impact of multiple T cell-APC encounters and the role of energy. *J. Comput. Appl. Math.*, 184(1):101–120, December 2005. ISSN 0377-0427.

[18] Lucy S. K. Walker and Abul K. Abbas. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nature Reviews Immunology*, 2(1):11–19, January 2002. ISSN

1474-1741. doi: 10.1038/nri701. URL http://www.nature.com/articles/nri701. Number: 1 Publisher: Nature Publishing Group.

[19] C. Kurts, H. Kosaka, F. R. Carbone, J. F. Miller, and W. R. Heath. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. *J Exp Med*, 186(2):239–245, July 1997. ISSN 0022-1007. doi: 10.1084/jem.186.2.239.

[20] Gary G. Singer and Abul K. Abbas. The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes in T cell receptor transgenic mice. *Immunity*, 1(5):365–371, August 1994. ISSN 1074-7613. doi: 10.1016/1074-7613(94)90067-1. URL http://www.sciencedirect.com/science/article/pii/1074761394900671.

[21] Rie Watanabe-Fukunaga, Camilynn I. Brannan, Neal G. Copeland, Nancy A. Jenkins, and Shigekazu Nagata. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature*, 356(6367):314–317, March 1992. ISSN 1476-4687. doi: 10.1038/356314a0. URL https://www.nature.com/articles/356314a0. Number: 6367 Publisher: Nature Publishing Group.

[22] Thorsten R. Mempel, Sarah E. Henrickson, and Ulrich H. von Andrian. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature*, 427(6970):154–159, January 2004. ISSN 1476-4687. doi: 10.1038/nature02238. URL https://www.nature.com/articles/nature02238. Number: 6970 Publisher: Nature Publishing Group.

[23] Mustapha Faroudi, Rossana Zaru, Pierre Paulet, Sabina Müller, and Salvatore Valitutti. Cutting Edge: T Lymphocyte Activation by Repeated Immunological Synapse Formation and Intermittent Signaling. *The Journal of Immunology*, 171(3):1128–1132, August 2003. ISSN 0022-1767, 1550-6606. doi: 10.4049/jimmunol.171.3.1128. URL

https://www.jimmunol.org/content/171/3/1128. Publisher: American Association of Immunologists Section: CUTTING EDGE.

[24] Sarah E. Henrickson, Thorsten R. Mempel, Irina B. Mazo, Bai Liu, Maxim N. Artyomov, Huan Zheng, Antonio Peixoto, Michael P. Flynn, Balimkiz Senman, Tobias Junt, Hing C. Wong, Arup K. Chakraborty, and Ulrich H. von Andrian. T cell sensing of antigen dose governs interactive behavior with dendritic cells and sets a threshold for T cell activation. *Nature Immunology*, 9(3):282–291, March 2008. ISSN 1529-2916. doi: 10.1038/ni1559. URL https://www.nature.com/articles/ni1559. Number: 3 Publisher: Nature Publishing Group.

[25] Byron B. Au-Yeung, Julie Zikherman, James L. Mueller, Judith F. Ashouri, Mehrdad Matloubian, Debra A. Cheng, Yiling Chen, Kevan M. Shokat, and Arthur Weiss. A sharp T-cell antigen receptor signaling threshold for T-cell proliferation. *PNAS*, August 2014. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.1413726111. URL https://www.pnas.org/content/early/2014/08/15/1413726111. Publisher: National Academy of Sciences Section: PNAS Plus.

[26] P. A. Reay, K. Matsui, K. Haase, C. Wulfing, Y. H. Chien, and M. M. Davis. Determination of the relationship between T cell responsiveness and the number of MHC-peptide complexes using specific monoclonal antibodies. *J Immunol*, 164(11):5626–5634, June 2000. ISSN 0022-1767. doi: 10.4049/jimmunol.164.11.5626.

[27] Francesco Marangoni, Thomas T. Murooka, Teresa Manzo, Edward Y. Kim, Esteban Carrizosa, Natalie M. Elpek, and Thorsten R. Mempel. The transcription factor NFAT exhibits signal memory during serial T cell interactions with antigen-presenting cells. *Immunity*, 38(2):237–249, February 2013. ISSN 1097-4180. doi: 10.1016/j.immuni.2012.09.012.

[28] Byron B. Au-Yeung, Heather J. Melichar, Jenny O. Ross, Debra A. Cheng, Julie Zikher-

man, Kevan M. Shokat, Ellen A. Robey, and Arthur Weiss. Quantitative and Temporal Requirements Revealed for Zap-70 Catalytic Activity During T Cell Development. *Nat Immunol*, 15(7):687–694, July 2014. ISSN 1529-2908. doi: 10.1038/ni.2918. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4095875/.

[29] Hélène D. Moreau, Gib Bogle, and Philippe Bousso. A virtual lymph node model to dissect the requirements for T-cell activation by synapses and kinapses. *Immunology & Cell Biology*, 94(7):680–688, 2016. ISSN 1440-1711. doi: https://doi.org/10.1038/icb.2016.36. URL https://onlinelibrary.wiley.com/doi/abs/10.1038/icb.2016.36. _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1038/icb.2016.36.

[30] R. J. De Boer and A. S. Perelson. How diverse should the immune system be? *Proc Biol Sci*, 252(1335):171–175, June 1993. ISSN 0962-8452. doi: 10.1098/rspb.1993.0062.

[31] Marie Le Borgne, Saravanan Raju, Bernd H. Zinselmeyer, Viet T. Le, JiaJia Li, Yingxiao Wang, Mark J. Miller, and Andrey S. Shaw. Real-Time Analysis of Calcium Signals during the Early Phase of T Cell Activation Using a Genetically Encoded Calcium Biosensor. *The Journal of Immunology*, 196(4):1471–1479, February 2016. ISSN 0022-1767, 1550-6606. doi: 10.4049/jimmunol.1502414. URL https://www.jimmunol.org/content/196/4/1471. Publisher: American Association of Immunologists Section: ANTIGEN RECOGNITION AND RESPONSES.

[32] Hiroaki Kitano. Biological robustness. *Nature Reviews Genetics*, 5(11):826–837, November 2004. ISSN 1471-0064. doi: 10.1038/nrg1471. URL https://www.nature.com/articles/nrg1471. Number: 11 Publisher: Nature Publishing Group.

[33] Nicholas S. Wilson, Dima El-Sukkari, Gabrielle T. Belz, Christopher M. Smith, Raymond J. Steptoe, William R. Heath, Ken Shortman, and José A. Villadangos. Most lymphoid organ dendritic cell types are phenotypically and functionally immature. *Blood*,

102(6):2187–2194, September 2003. ISSN 0006-4971. doi: 10.1182/blood-2003-02-0513. URL https://doi.org/10.1182/blood-2003-02-0513.

[34] Stéphanie Hugues, Luc Fetler, Laura Bonifaz, Julie Helft, François Amblard, and Sebastian Amigorena. Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity. *Nature Immunology*, 5(12):1235–1242, December 2004. ISSN 1529-2916. doi: 10.1038/ni1134. URL https://www.nature.com/articles/ni1134. Number: 12 Publisher: Nature Publishing Group.

[35] T. W. McKeithan. Kinetic proofreading in T-cell receptor signal transduction. *PNAS*, 92(11):5042–5046, May 1995. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.92.11.5042. URL https://www.pnas.org/content/92/11/5042. Publisher: National Academy of Sciences Section: Research Article.

[36] Jan Paul Medema, Danita H. Schuurhuis, Delphine Rea, Joost van Tongeren, Joan de Jong, Sandra A. Bres, Sandra Laban, René E.M. Toes, Mireille Toebes, Ton N.M. Schumacher, Bellinda A. Bladergroen, Ferry Ossendorp, J. Alain Kummer, Cornelis J.M. Melief, and Rienk Offringa. Expression of the Serpin Serine Protease Inhibitor 6 Protects Dendritic Cells from Cytotoxic T Lymphocyte–Induced Apoptosis. *J Exp Med*, 194(5):657–668, September 2001. ISSN 0022-1007. URL https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2195949/.

[37] Mark S. Anderson, Emily S. Venanzi, Zhibin Chen, Stuart P. Berzins, Christophe Benoist, and Diane Mathis. The cellular mechanism of Aire control of T cell tolerance. *Immunity*, 23(2):227–239, August 2005. ISSN 1074-7613. doi: 10.1016/j.immuni.2005.07.005.