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Immunological self-tolerance: Lessons from mathematical modeling

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Abstract

One of the fundamental properties of the immune system is its capacity to avoid autoimmune diseases. The mechanism underlying this process, known as self-tolerance, is hitherto unresolved but seems to involve the control of clonal expansion of autoreactive lymphocytes. This article reviews mathematical modeling of self-tolerance, addressing two specific hypotheses. The first hypothesis posits that self-tolerance is mediated by tuning of activation thresholds, which makes autoreactive T lymphocytes reversibly “anergic” and unable to proliferate. The second hypothesis posits that the proliferation of autoreactive T lymphocytes is instead controlled by specific regulatory T lymphocytes. Models representing the population dynamics of autoreactive T lymphocytes according to these two hypotheses were derived. For each model we identified how cell density affects tolerance, and predicted the corresponding phase spaces and bifurcations. We show that the simple induction of proliferative anergy, as modeled here, has a density dependence that is only partially compatible with adoptive transfers of tolerance, and that the models of tolerance mediated by specific regulatory T cells are closer to the observations.

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Keywords: Self-tolerance; Tuning; Regulatory CD4+CD25+T cells; Modeling

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1. Introduction

Mathematical modeling of the immune system often concentrates on the immune responses to pathogens. This article deals with another fundamental process in the immune system: the maintenance of *self-tolerance*, i.e., the prevention of harmful immune responses against body components. The biological significance of this process becomes very patent upon its failure during pathological conditions known as autoimmune diseases.

The risk of autoimmunity cannot be dissociated from the capacity of the immune system to cope with diverse and fast evolving pathogens [28]. The latter is achieved by setting up a vast and diverse repertoire of antigen receptors expressed by lymphocytes, which as a whole is capable of recognizing any possible antigen. Most lymphocytes have a unique antigen receptor (immunoglobulin in B-cells and TCR in T cells) that is encoded by a gene that results from somatic mutation and random assortment of gene segments in lymphocyte precursors. The randomness in the generation of antigen receptors makes it unavoidable that lymphocytes with receptors recognizing body antigens are also made. These *autoreactive* lymphocytes can potentially cause autoimmune diseases if their activation and clonal expansion are not prevented. The question is how is this avoided in healthy individuals?

According to Burnet's original clonal selection theory [6] expansion of autoreactive lymphocytes and autoimmunity would be avoided by deleting the autoreactive lymphocytes from the repertoire once and for all during embryonic development. The fact that the generation of lymphocytes is a life long process in mammals invalidated this possibility. Following an early suggestion by Lederberg [29] deletion of potentially self-destructive lymphocytes was reformulated as an aspect of lymphopoiesis. Accordingly, lymphocytes that express an autoreactive receptor are deleted at an immature stage of their development [25,1], before they can undergo clonal expansion and trigger destructive immune responses.

But deletion alone cannot explain self-tolerance. The major shortcoming of deletion models of self-tolerance is the well-documented presence of mature autoreactive B and T lymphocytes in normal healthy animals [39]. Many different experiments have demonstrated that these autoreactive T cells can undergo clonal expansion and cause disease. In this paper we will focus on a particular type of experiments first reported by Sakaguchi and coworkers [41,42], which allows assessing self-tolerance from the perspective of the population dynamics of circulating lymphocytes. CD4+ T cells were isolated from healthy animals and subsets of this population were transferred into syngeneic recipient animals, which were devoid of T cells. Transfer of CD4+CD25- T cells resulted in the expansion of these cells in the recipients and caused an autoimmune syndrome characterized by multiple organ-specific autoimmune diseases (illustrated in Fig. 1). These results indicate that in the healthy individuals there are significant numbers of autoreactive cells that could potentially proliferate and mount deleterious immune responses to self.

How are those autoreactive T cells, circulating in healthy individuals, prevented from mounting harmful immune responses against body tissues? There are several hypotheses in the literature (see the special issue of *Seminars Immunology* (vol 12 issue 3) for a rather comprehensive overview). One hypothesis posits that autoreactive T cells are prevented from proliferating and mounting immune responses because specific regulatory T cells control them. In the above-mentioned Sakaguchi et al. experiment (Fig. 1), those animals devoid of T cells receiving the same number of CD4+CD25+ T cells or receiving equal numbers of CD4+CD25- and CD4+CD25+ T cells did not develop autoimmune diseases. Prevention of autoimmunity in the recipients by transfer of CD25+ T cells suggests the existence of regulatory T cells within the CD25+ subset, which exert a direct suppressive interaction on CD25- T cells. Although this interpretation has been favored by immunologists, recent evidence suggests that competition and

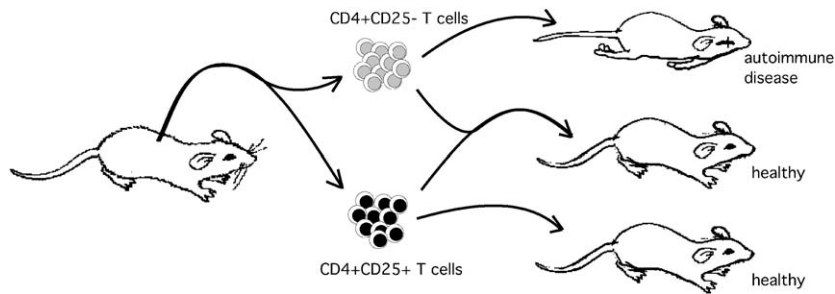


Fig. 1. Illustration of the experiments of Sakaguchi et al. demonstrating the existence of autoreactive T cells in healthy individuals. Purified CD4+CD25- T cells, but not CD4+CD25+ T cells, from healthy animals will cause autoimmune diseases in recipient animals. An interaction between the two subsets of CD4 cells prevents disease development in the same recipients.

density-dependent inhibition of cell expansion in recipients may be sufficient to explain the inhibitory effects of CD4+CD25+ cells on CD4+CD25- cells [4], and thus postulating direct suppressive effects could be superfluous. Another hypothesis for the prevention of harmful immune responses by autoreactive T cells is that these cells become unresponsive to self-antigens by modification of their cell-signaling machinery; immunologists use the word “anergy” to refer to this unresponsiveness of cells, namely when this is reflected in diminished proliferative responses [45]. Among the possible explanations for self-specific anergy induction, perhaps the simplest is the hypothesis that lymphocytes tune up their activation thresholds in response to recurrent stimuli [17–20]. According to this tunable activation threshold (TAT) hypothesis, autoreactive lymphocytes that are frequently stimulated by particular self-antigens adapt to the recent time-average of such stimulation so that they fail to be activated by these antigens.

We have previously addressed these two hypotheses by modeling the population biology of autoreactive T lymphocytes. In this article we review our published results and present new ones obtained with two simplified models of autoreactive T cell dynamics. Basically, we ask here whether and to what extent the underlying mechanisms of tolerance induction and maintenance within each model are compatible with the basic aspects of the Sakaguchi phenomenon (Fig. 1). This phenomenon is particularly suitable for modeling since in these experiments the control of autoimmunity can be understood as the control of proliferation, in the absence of thymic influx that would otherwise complicate the mathematics. In the next section we propose and analyse a hypothesis according to which recurrent stimulation by self-antigens and tuning of activation thresholds regulate the proliferative responses of autoreactive T lymphocytes. We show that this induction of proliferative anergy in autoreactive T cells has a density dependence that is only partially compatible with the Sakaguchi phenomenon. In contrast, as shown in Section 3, our model of regulation of activation-dependent proliferation of autoreactive T cells by specific regulatory T cells results in realistic density dependence.

2. Modeling tolerance by tuning of activation thresholds of individual T lymphocytes

The tunable activation threshold (TAT) hypothesis by Grossman et al. [17–20] proposes that every interaction between the TCR and its ligand on antigen presenting cells (APCs) results in an intracellular competition between “excitation” and “de-excitation” signaling pathways that causes the T cell to adapt

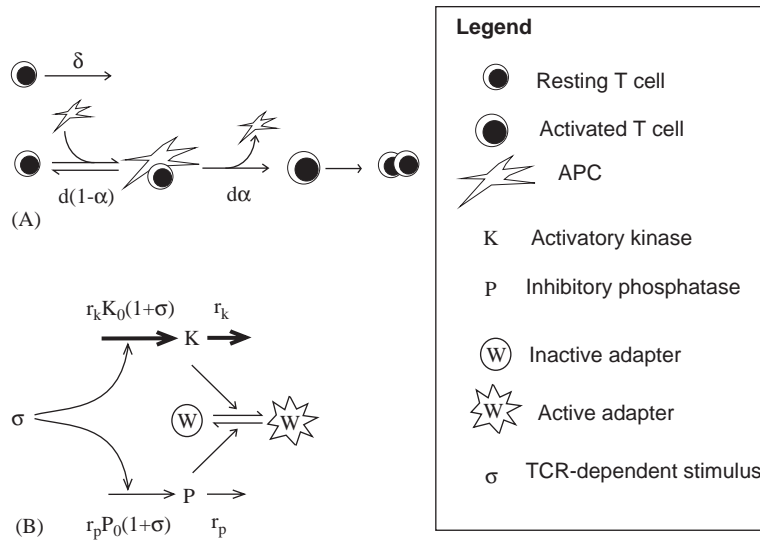


Fig. 2. Illustration of a model of the population dynamics of lymphocytes with tunable activation thresholds indicating the cellular processes (A) and the molecular processes of the cell machinery (B).

to the stimulation by increasing or decreasing its threshold for activation. Recently, this hypothesis has been discussed by other authors in the context of peripheral tolerance, and T cell proliferation and homeostasis [38,48,53,58]. Mathematical models of adaptation of neural synapses would be a straightforward inspiration for modeling the TAT mechanism. However, the set up of the immune system poses specific problems. In neural tissue, intermittent signaling does not depend on de novo formation of synapses, which are sessile. In contrast, the immunological synapses are intermittent and their formation depends on the relative densities of T cells and APCs bearing agonist antigens. Synapse formation and signaling is therefore coupled to population dynamics, which, as we will see below, poses novel mathematical modeling problems. Grossman and Paul [17,19] extensively discuss the coupling of tuning and population dynamics but an explicit mathematical model was not put forward. The model presented here features tuning of the signaling machinery of individual T cells in close agreement with what was proposed by Grossman and Paul [19]. However, the way this signaling is coupled to the population dynamics departs from the proposal of these authors. Here, adaptation is restricted to the activation-dependent proliferative response, whereas the general tuning hypothesis by Grossman and colleagues is more comprehensive, contemplating features such as the role of suppression in inducing tuning and vice-versa, and tuning of APCs. In fact, Grossman and colleagues did not propose that TAT per se would be responsible for regulating the expansion and maintenance of T cell populations as we are studying here.

2.1. A minimal model

Activation, proliferation and survival of T lymphocytes require recurrent interactions of their TCRs with their ligands, the MHC-peptide complexes, at the membrane of APCs [57,40]. This APC-dependent population dynamics is captured in the reactional diagram proposed by De Boer and Perelson [9] (Fig. 2A). Assuming that the densities of the conjugated T cells are in quasi-steady state [9,49], this

diagram is translated into the following differential equation (see Appendix A for derivation):

$$\frac{dT}{dt} = d\alpha C - \delta(T - C), \tag{1}$$

where T is the T cell density, d is the conjugate dissociation rate, δ is the per cell death rate, and C is the quasi-steady state conjugate density:

$$C \cong \frac{c(T + A) + d - \sqrt{-4 \cdot A \cdot T \cdot c^2 + (c(T + A) + d)^2}}{2c}. \tag{2}$$

According to the TAT hypothesis, continuous signaling by the TCR would lead to dynamic adaptation of the signal transduction machinery. To incorporate this in the model above we must define the probability α of productive conjugation as a function of the signaling machinery status of the conjugated lymphocyte. Following closely the conceptual signaling model by Grossman and Paul [18,19], Sousa et al. [49] assumed that T cell activation is controlled by a “futile cycle” downstream of TCR signaling, involving a kinase and a phosphatase that operate on an adapter molecule [7,15,50] (Fig. 2). Also as suggested by Grossman and Paul [17,19], it was assumed that the phosphorylation state of the adapter is hypersensitive to the relative activities of the two enzymes and behaves as a molecular switch [26]. All the adapter molecules are phosphorylated if the kinase activity is higher than that of the phosphatase; otherwise, the adapter is fully dephosphorylated.¹ During lymphocyte conjugation with an APC, TCR stimuli result in a faster increase in kinase and phosphatase activities. The lymphocyte will be activated and enter cell cycle if after conjugating with the APC the kinase activity supersedes that of the phosphatase, and it will remain quiescent otherwise. Note that by linking activation to the function of proliferation alone we depart from Grossman and Paul [19], who discuss other functions relevant to population dynamics as well. The dynamics of this signaling machinery was represented by two differential equations:

$$\frac{dK}{dt} = r_K(K_0(1 + \sigma) - K), \tag{3}$$

$$\frac{dP}{dt} = r_P(P_0(1 + \sigma) - P), \tag{4}$$

where K is the kinase activity, P is the phosphatase activity, r_K is the turnover rate of the kinase, r_P is the turnover rate of the phosphatase, K_0 is the basal steady state kinase activity, and P_0 is the basal steady state phosphatase activity. Parameter σ is the magnitude of the stimulus to the kinase and phosphatase production rates, which takes the value 0 if the cell is free and σ if conjugated. This signaling cascade shows adaptive properties [19] provided that the turnover rate of the kinase is higher than that of the phosphatase ($r_K > r_P$), and that, for any stimuli, the steady state activity of the phosphatase is higher than that of the kinase ($P_0 > K_0$). Under these conditions, the adapter can be transiently switched on, but it will be switched off eventually if the stimulus persists.

This simple mathematical TAT model was developed and analysed in Sousa et al. [49], based mainly on Monte-Carlo stochastic simulations of individual cells. In this article we present a further simplification, which is amenable to analytic treatment and retains the main properties of the original model. This simplification involves two additional approximations. First, we assume that turnover of the kinase activity

¹ A very similar adaptation model was developed and analysed by Levchenko and Iglesias [36] in the context of gradient sensing.

is very fast as compared to the conjugate dissociation rate ($r_K \gg d$), and as compared to the turnover rate of the phosphatase activity ($r_K \gg r_P$). Under these conditions, the kinase activity is in quasi-steady state, and can be approximated by either $K = K_0(1 + \sigma)$ or $K = K_0$, when the T lymphocyte is conjugated to an APC, resulting in a stimulus σ or when the lymphocyte is free, resulting in no stimulus, respectively. The second approximation consists of assuming that for any given density of T cells and APCs, the fast conjugation and deconjugation processes are practically in equilibrium. This implies that the probability density functions (PDFs) of the phosphatase activity in conjugated and in free T cell populations are stationary. These approximations were mainly motivated by the simplicity they confer to the mathematics. The first assumption can be biologically sustained since very early events seem to define whether or not a cell will be activated. As for the second assumption, it is not guaranteed that it holds in a growing population since it is unlikely that the distribution of the phosphatase activity will become stationary before the size of the population changes. However, it will obviously hold at the equilibrium, and therefore it is safe to draw qualitative conclusions from this simplified model in terms of number and stability of its steady states. Our confidence is further supported by the fact that the same qualitative results were obtained with more realistic Monte-Carlo simulations of individual cells where these simplifying assumptions were not introduced [49].

Milutinovic et al. [37] used stochastic hybrid-automaton theory to describe the PDFs of cell-associated molecules in cells that cycle between APC-conjugation and APC-free states. The dynamics of phosphatase PDF in conjugated and free T cells, respectively ρ_C and ρ_F , are described by the following set of first-order partial differential equations:

$$\frac{\partial \rho_C}{\partial t} + \frac{\partial}{\partial P}(P_C \rho_C) = -d \rho_C + c_E \rho_F, \quad (5)$$

$$\frac{\partial \rho_F}{\partial t} + \frac{\partial}{\partial P}(P_F \rho_F) = d \rho_C - c_E \rho_F, \quad (6)$$

where P_C and P_F are the functions governing the dynamics of the phosphatase in the conjugated and free regimes (i.e. the right-hand side of Eq. (4) with $\sigma > 0$ and $\sigma = 0$, respectively $r_P(P_0(1 + \sigma) - P)$ and $r_P(P_0 - P)$), and c_E and d are the transition rates from the free to the conjugated state ($c_E = c(A - C)$) and from the conjugated to the free state, respectively.

Assuming that the conjugation and deconjugation processes are in quasi-steady state, we expect the time derivatives to vanish. Under these conditions, we obtain the following equation:

$$\frac{\partial}{\partial P}(P_C \rho_C + P_F \rho_F) = 0 \quad (7)$$

which, as demonstrated by Milutinovic et al. [37], can be used to reduce the system to the following differential equation:

$$\frac{\partial}{\partial P}(P_C \rho_C) = - \left(d + c_E \frac{P_C}{P_F} \right) \rho_C. \quad (8)$$

The solution of this equation is

$$\rho_C = \begin{cases} N |P_0(1 + \sigma) - P|^{(d/r_P)-1} |P_0 - P|^{c_E/r_P}, & P_0 \leq P \leq P_0(1 + \sigma), \\ 0 & \text{else,} \end{cases} \quad (9)$$

where N is a normalisation constant (a detailed derivation of Eq. (9) is provided in Appendix B).

The fraction α of T cells that is activated and divides when conjugation ceases (i.e. the fraction of T cells that at the instant of releasing from the APC have $K > P$) is then:

$$\alpha = \int_{P_0}^{K_0(1+\sigma)} \rho_C dP. \tag{10}$$

Substituting this definition in Eq. (1) we fully define the population dynamics of the T cells with TAT.

2.2. Results

The (K, P) -signaling machinery shows an activation threshold that is tunable. It is easy to note that if the value of the phosphatase activity at the beginning of conjugation is $P \geq K_0(1 + \sigma)$ then this is sufficient (although not necessary) to prevent activation of the T cell. The threshold is modulated by the history of stimuli to the T cell, which determines the value of the phosphatase activity P at any given time. Therefore, from the point of view of the population biology of T cells, in this model, as in the conceptual model of Grossman and Paul [17], the activation threshold is dependent on the frequency of interactions of T cells with the APCs, i.e. the frequency of the stimuli to the individual T cells [49] (Fig. 3A). If T cells were always free, the probability density function of the phosphatase activity would correspond to a Dirac Delta at P_0 ; whereas if all the lymphocytes were permanently conjugated to APCs delivering the same stimulus σ then the PDF would be a Dirac Delta at $P_0(1 + \sigma)$ (Fig. 3B). Since T cells are cycling between conjugation and free periods, the stationary PDF of P in the population takes values in the interval $[P_0, P_0(1 + \sigma)]$ (Appendix B).

The frequency of APC interactions per T cell decreases as T cell density increases due to competition (Eq. (2)). This implies that, as T cell density increases, the median of the PDF of phosphatase activity in conjugated T cells ($\rho_C(P)$) becomes closer to the value P_0 ; reciprocally, as T cell density decreases, the median of the PDF approaches $P_0(1 + \sigma)$ (Fig. 3B). This means that the fraction of cells α undergoing productive conjugation to activation and cell cycle increases with T cell density. This defines a positive feedback loop such that increases (decreases) in T cell density result in higher (lower) average values of α , which lead to further increases (decreases) in T cell density. This positive feedback loop resulting from the present implementation of tunable activation thresholds is the opposite of a density-dependent feedback population control. In our model this loop interacts with the negative feedback loop defined by the effect of competition on the density of conjugates. For this reason, the model has two possible stable steady states: one in which lymphocyte population is extinct and one in which it is limited by APC availability, and predominantly made of nonanergic lymphocytes (Fig. 3C). The bifurcation diagram (Fig. 3D) of the steady state population size as a function of the ratio P_0/K_0 , which is a measure of the adaptability of the signaling cascade, indicates that the main contribution of tunable thresholds at intermediate P_0/K_0 values is to change the size of the basins of attraction of the extinction and APC-limited states by shifting the position of the saddle point (actually in a model without adaptation there is no saddle point and the extinction state is unstable [9,49]); as this control parameter increases the size of the population in the saddle point, and decreases that of the APC-limited state, there is a fold-bifurcation at a critical value of this parameter in which both points merge into a single one. Beyond this critical value such points disappear. In this mathematical TAT model the only way the population of autoreactive T cells can persist is by competition for limited numbers of APCs; if TAT effects predominate the population will become extinct.

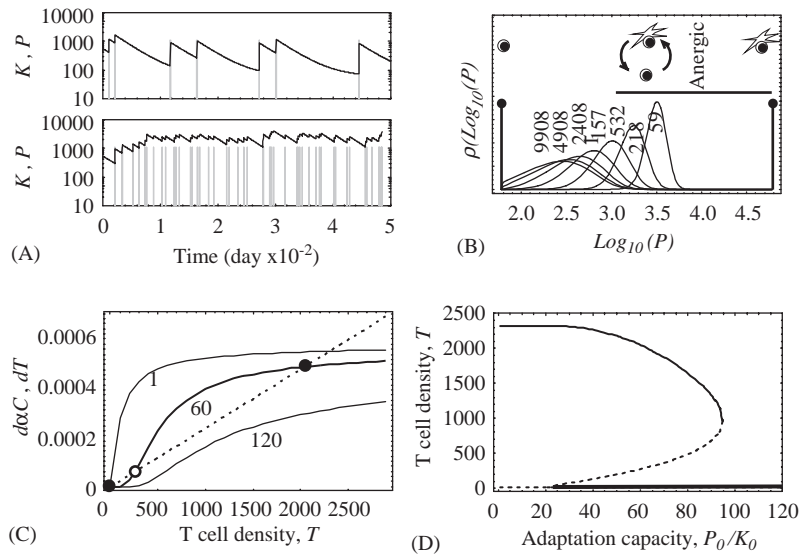


Fig. 3. Analysis of the model of the population dynamics of T cells with tunable activation thresholds. (A) Kinetics of the phosphatase (black line) and the kinase (gray line) which are downstream of TCR stimulus in an individual T cell. The probability that the phosphatase activity P supersedes the kinase activity K at the instant of deconjugation increases with the frequency of encounters. (Top: $c(A-C) = 0.20$; bottom: $c(A-C) = 0.048$). (B) Stationary probability density function of the phosphatase activity P in the population of conjugated T cells at the indicated densities ($P_0 = 60$; $\sigma = 1000$). (C) Phase diagram of the model indicating the death rate (dashed line) and growth rates (solid lines) for the indicated values of the control parameter P_0/K_0 ; the dots indicate the stable (black) and unstable (white) steady states for the reference value $P_0/K_0 = 60$. (D) Bifurcation diagram obtained by varying the control parameter P_0/K_0 that determines the adaptation capacity of the signaling machinery; Stable and unstable steady states are indicated by solid and dashed lines respectively. (Reference parameters: $r_p = 0.027 \text{ day}^{-1}$; $K_0 = 1 \text{ au}$; $P_0 = 60 \text{ au}$; $\sigma = 1000$; $A = 8 \text{ cells}$; $d = 6 \text{ day}^{-1}$; $c = 0.06 \text{ cell}^{-1} \text{ day}^{-1}$; $\delta = 0.02 \text{ day}^{-1}$; $\text{au} = \text{relative activity units}$).

2.3. Specific discussion

Our model in which the TAT-signaling machinery is coupled to the growth dynamics of T cell populations offers a mechanism of self-tolerance by prevention of autoreactive T cell expansion, and their eventual deletion from the circulating pool. Therefore, according to this model, the persistence of circulating anergic T cells requires their continuous influx from the thymus, as demonstrated by Sousa et al. [49]. This is not unreasonable because the thymus continuously produces T cells although at a rate that decreases with age. However, when confronted with the Sakaguchi phenomenon, which involves adoptive transfers of peripheral T cells in the absence of thymic influx, the present mathematical model shows some important limitations.

Within the framework of our model, an adoptive transfer procedure in which lymphocytes are isolated *ex vivo* can be interpreted as an extra time-period during which the transferred lymphocytes remain free from the APCs. This manipulation would increase the responsiveness of lymphocytes as compared to the steady state *in vivo*, and this would be compatible with the experimental observations that CD4+CD25- lymphocytes from healthy subjects can induce autoimmunity in empty recipients. The fact that CD4+CD25+ T cells do not induce autoimmune disease in the recipient animals can also be interpreted by assuming that CD25+ cells have higher thresholds of activation (higher values of phosphatase activity P) than

CD25⁻ cells. This is not unreasonable because it is well documented that, following anergy induction in vitro, lymphocytes upregulate the CD25 molecule [27]. However, the observation that cotransfers of CD4⁺CD25⁻ and CD4⁺CD25⁺ cells result in tolerance cannot be interpreted within the framework of our simple mathematical TAT model alone. As we have demonstrated, in our model “more cells should lead to more responsiveness or less anergy”, and therefore adding CD25⁺ T cells to CD25⁻ cells should have increased the proliferative responsiveness of the CD25⁻ cells rather than suppress it and change the total population steady state as observed [3]. The result of the co-transfers thus point to the existence of some kind of interaction between cell populations that was not taken into account in our simple mathematical model. A suppressive interaction among T cells will be explicitly discussed and modeled in the next section.

As mentioned above, the model we presented here was designed to include the properties of tuning of activation thresholds posited by Grossman and Paul [19] but, by coupling tuning to the proliferative response alone, it departs significantly from the more comprehensive conceptual hypothesis that these authors have put forward. The dependence of TAT on the frequency of T cell-APC encounters has been qualitatively described by Grossman and Paul [17,19], who discussed that antigen-stimulated expansion of T cells is regulated through the combined action of T-cell tuning and of complementary cell-density effects [19,16]. Thus, Grossman and Paul [17] suggested that tuned T-cells would suppress other T cells by raising their activation thresholds. Furthermore, they suggested [17] that tuning would apply to APCs as well, and hypothesized that the ability of APCs to stimulate T cells could be down-regulated as the frequency of encounters with T cells increases. Therefore, our results are in line with the general qualitative views of Grossman and colleagues.

3. Modeling tolerance mediated by regulatory CD4⁺CD25⁺ T lymphocytes

In recent years, a lot of information has been gathered on regulatory T cells within the CD4⁺CD25⁺ pool (for a review see [42]). These cells seem to be produced already differentiated in the thymus [43,5,24]. They present unique transcription factors that confer them the regulatory phenotype [22]. Their expansion and persistence in the periphery is dependent on recurrent interactions with APCs, which present self-antigens [46,14]. Regulatory T cells do not produce autocrine growth factors, namely IL-2 [52,54]. Lafaille and colleagues [13] have provided evidence that in vivo regulatory T cell populations require IL-2 produced by other cells. We have demonstrated on theoretical grounds that regulatory T cells must use the T cells they suppress “as growth factor” [33,34]. Regulatory T cells may promote the differentiation of their targets into the regulatory phenotype, or use a growth factor produced by their targets. We provided experimental evidence for this latter possibility in vitro [30].

3.1. A minimal model

As in the previous section, our model follows the dynamics of a population of autoreactive T cells whose activation, proliferation and survival depends on interactions with a homogeneous population of APCs. This T cell population is made of two subpopulations of regulatory (T_R) and effector (T_E) cells, with the same antigenic specificity. T_E cells are responsible for autoimmune disease if T_R cells do not control their activation-dependent expansion. The diagram in Fig. 4A illustrates the basic processes in the model. Briefly, resting T_R and T_E cells can die or form conjugates with free sites on the APCs. Conjugation can

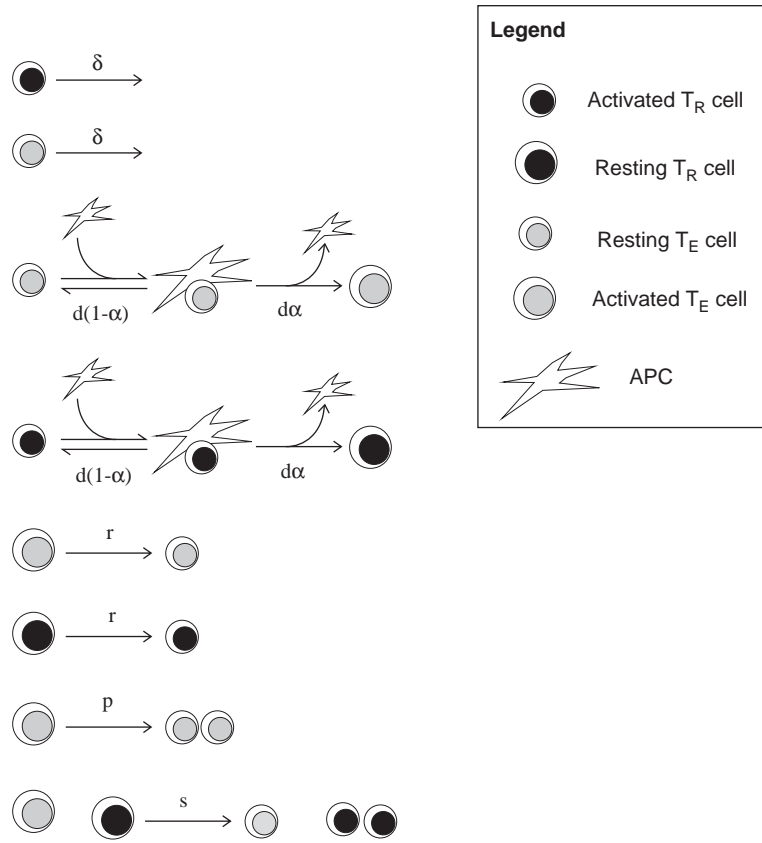


Fig. 4. Illustration of the regulatory T cell population model indicating all the processes in which APCs, T_E and T_R cells are involved.

be productive, resulting in T cell activation, or nonproductive such that the T cell remains in resting state. T cell activation is transient, and activated T cells will spontaneously rest. Activated T_R and T_E cells, but not resting cells, will mutually interact. Activated T_E cells, but not T_R cells or resting T_E cells, produce a growth factor. The growth factor acts on the T_E producing it in an autocrine way, and on other activated T_E and T_R cells on a paracrine way. Activated T_R cells and T_E cells divide as a function of this growth factor. The activation of a T_E cell is inhibited upon interaction with an activated T_R cell. T_R cells do not produce growth factors.

The mechanism underlying mutual interactions between T_R and T_E cells is a major issue. Leon et al. [31–34] have proposed and analysed a model in which mutual interactions between T cells require their simultaneous conjugation with an APC, i.e. the formation of multicellular conjugates. This mechanism is in accordance with the dependence of in vitro suppression on the ratio between T_R cell and APC numbers [34]. However, Thornton and Shevach [55] have shown that T_R cells, which have been previously activated by APCs, may suppress T_E cells by direct cell-to-cell contact in vitro. The results illustrated in the present article were obtained with a model assuming direct interactions between activated T_R and T_E cells. When appropriate, we will pinpoint the differences with the Leon et al. model. From the outset it is important

to note that in the Leon et al. model, efficient suppression of T_E cells requires the presence of a minimum number of T_R cells *per APC* [34], while efficient suppression in the model used here merely requires a minimum density of T_R cells irrespective of the number of APCs. The qualitative differences between the two models unfold from these different postulates about APC-dependence.

Assuming that the densities of conjugates, and of activated T cells are in quasi-steady state, the reactional diagram in Fig. 4 can be translated into the following pair of ordinary differential equations (see derivation in Appendix C):

$$\frac{dR}{dt} = sE_A R_A - \delta R, \tag{11}$$

$$\frac{dE}{dt} = pE_A - \delta E, \tag{12}$$

with

$$R_A = \frac{\alpha d R_C}{r + sE_A}, \tag{13}$$

$$E_A = - \frac{r(p+r) + ds\alpha(R_C - E_C) - \sqrt{4d(p+r)rs\alpha E_C + (-r(p+r) - ds\alpha(R_C - E_C))^2}}{2(p+r)s}, \tag{14}$$

$$E_C = \frac{EA}{\frac{d}{c} + R + E}, \tag{15}$$

$$R_C = \frac{RA}{\frac{d}{c} + R + E}, \tag{16}$$

where R and E are the total density of T_R and T_E cells, A is total density of APC-conjugation sites (assumed to be constant), c is the conjugation rate, d is the deconjugation rate, δ is the death rate, s is the suppression rate, and r is the reversion rate of an activated T cell to the resting state. This model is highly nonlinear, and since we could not obtain closed expressions for the steady states, we made numerical phase-plane and bifurcation analyses.

Like in the Leon et al. model [33], the richest phase-plane of this (R, E) model has 4 steady states, and displays bistability (Fig. 5A). The steady states are: the trivial $(0,0)$ state, corresponding to the extinction of T_R and T_E cells, which is unstable; an unstable saddle-point where both T_R and T_E coexist, (R_3, E_3) ; a stable state of coexistence of T_R and T_E cells, (R_2, E_2) ; and another stable state in which T_R cells are competitively excluded by T_E cells, $(0, E_1)$. Following [33], we interpret the stable coexistence of T_R and T_E cells as *self-tolerance* and the competitive exclusion of T_R cells by T_E cells as *autoimmunity*.

The (co-)existence of these steady states in the phase-plane is controlled by the relative values of the parameters determining the net growth of the T_R population (d, K, A , and s), and the net growth of the T_E population (d, K, A , and p). Relative high net growth of the T_R population as compared to T_E leads to a global stability of the self-tolerance state; while relative low growth of T_R cells results in disappearance of the self-tolerance state and global stability of the autoimmunity state.

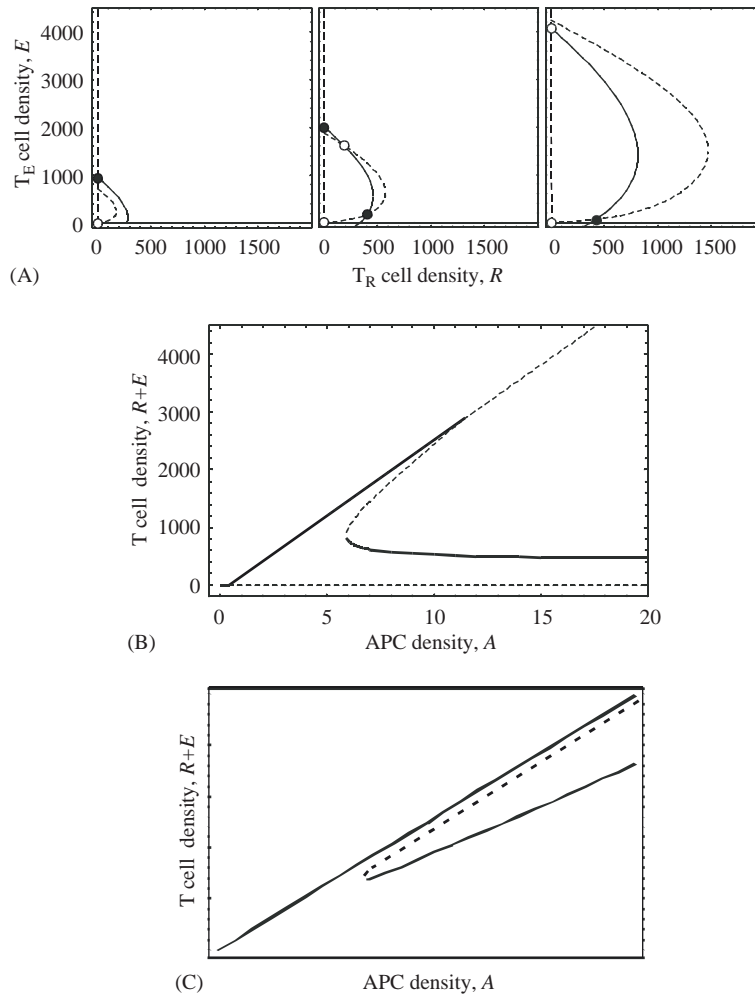


Fig. 5. Phase planes for the regulatory T cell population model, and bifurcation as a function of the APC density. (A) and (B) The model presented in the main text was used with parameters: $p = 2 \text{ day}^{-1}$, $r = 0.3 \text{ day}^{-1}$, $d = 6 \text{ day}^{-1}$; $c = 0.06 \text{ cdu}^{-1} \text{ day}^{-1}$; $\delta = 0.02 \text{ day}^{-1}$; $\alpha = 1$ and $s = 0.07 \text{ cdu}^{-1} \text{ day}^{-1}$; $\text{cdu} =$ relative cell density units. The three phase diagrams in (A) were obtained from left to right with $A = 5, 10$ and 15 cdu respectively. (C) Bifurcation diagram for the model described by Leon et al. [32].

One important control parameter is the density of APCs, A . In Fig. 5B we present a typical bifurcation diagram of total density of T_R and T_E ($R + E$) as a function of the value of A . Too low densities of APCs are unable to sustain any T cells in the population. The state $(0,0)$ is globally stable and the state $(0, E_1)$ is unstable and has no physical meaning because $E_1 < 0$. Following a transcritical bifurcation involving these two steady states, the state $(0, E_1)$ becomes stable and also gains physical meaning ($E_1 \geq 0$) (a representative phase plane is depicted in Fig. 5A-left). For an interval of relatively low values of A only T_E cells can be sustained in the population. For higher values of A , following a fold-bifurcation that brings in the unstable saddle (R_3, E_3) and a stable state (R_2, E_2) , the system becomes bistable, such that depending on initial conditions either the autoimmunity or the tolerance states can be reached (representative phase

plane in Fig. 5A-middle). For even higher values of A there is another transcritical bifurcation involving the unstable saddle (R_3, E_3) and the competitive exclusion state $(0, E_1)$. The latter state becomes unstable and the previously unstable coexistence state becomes stable but physically meaningless because R_3 is now negative (i.e. the two nontrivial nullclines intersect in the quadrant $(R < 0, E > 0)$). As a consequence of this bifurcation, the state of coexistence of T_E and T_R cells (R_2, E_2) becomes the only stable state with physical meaning. This is a major difference between the model presented here and the model of Leon et al. [33], where the increase of A never results in a bifurcation from the bistability to the global stability regimes (Fig. 5C). In the present model, as the APC density increases the size of the population at the coexistence steady state, $E + R$, tends asymptotically to a constant value (suppression requires a minimal density of T_R cells) (Fig. 5B). In the Leon et al. model [33], $E + R$ in the coexistence state increases linearly with the density of APCs (suppression in the presence of more APCs requires more T_R cells to “cover” the same fraction of APCs) (Fig. 5C).

3.2. Specific discussion

The (R, E) model presented here and the model proposed and studied by Leon et al. [33] offer a mechanism of self-tolerance by prevention of autoreactive T cell expansion. These two models can readily explain the Sakaguchi phenomenon. In healthy individuals the subpopulation of CD4+CD25- T cells is enriched in T_E cells, while the subpopulation of CD4+CD25+ T cells is enriched in T_R cells. The fraction of T_E cells is so high in the first CD4 subset that its transfer into empty animals results in autoimmunity; while the fraction of T_R cells in the second CD4 subset is sufficiently high that when mixed with the first subset leads to tolerance. Several authors [3,2,21] have analysed the population dynamics of CD4+CD25+ and CD4+CD25- T cells in recipient animals, showing that the presence of regulatory T cells reduces the apparent steady state density of CD25- T cells. They also reported that when transferred alone CD4+CD25+ T cells expand and persist in the recipients [3,2,21], suggesting that this subset is an impure population of T_R cells containing also T_E cells (which act as a source of growth factors), or that T_R cells obtain growth factors also from non-T cells. However, the number of CD25+ T cells recovered are higher in the presence of CD25- (Demengeot, personal communication), confirming our theoretical results according to which CD25- act as a source of growth factors, albeit non-T cell derived growth factors might be also present.

The present (R, E) model retains several immunologically meaningful properties previously identified in the Leon et al. model. For example, it predicts that diverse subclonic infections will have a net protective effect against autoimmunity [31]. However, the Leon et al. model, but not the present (R, E) model, predicts a strong impact of changes in APC density on the steady state attained within the bistability regime: a relatively fast increase in APC density will force a switch from the self-tolerance to the autoimmunity state. This switch from one stable state to another requires an APC-dependent suppressive interaction, and is not recovered with the (R, E) model presented here, which features a direct T_R - T_E interaction. This switch is biologically meaningful, since it provides a common rationale for the etiology of some autoimmune diseases that are associated to specific infections [31], for the ability to cause experimental autoimmune pathologies through immunization with self-antigens in adjuvants [33], and for the ability to experimentally induce autoimmunity by neonatal thymectomy [33,32]. None of these properties are recovered in the simple (R, E) model presented here. Therefore we conclude that the Leon et al. model might be capturing better the reality of self-tolerance mediated by regulatory T cells in vivo.

4. General discussion

This article reviewed mathematical models of self-tolerance by control of expansion of autoreactive T cell populations mediated by two mechanisms: tunable activation thresholds without suppression or suppression by regulatory T cells without tuning. We have shown that proliferative energy in our simple TAT model decreases with T cell density relative to APCs. Due to this property, the model can explain efficient control of expansion of autoreactive T cells, but not their persistence. In the second model, the existence of a tolerance steady-state in which T_R and T_E cells coexist is compatible with the fact that from every self-tolerant individual autoreactive T cells can be purified that can cause autoimmunity. In contrast with the simple TAT model analyzed here, this will be true even in the absence of a continuous influx of cells from the thymus. Extrapolating from these examples, it is clear that models that can explain the equilibrium between cell proliferation and death, in the absence of external sources, must include some form of T cell-density dependent suppression. As noted earlier, some conceptual models have postulated an interplay between suppression and tuning of activation thresholds [19,16], but the involvement of the later regulatory mechanism remains to be established. Our results indicate that suppression mediated by regulatory T cells is sufficient to explain the prevention of pathologic autoimmune responses by effector T cells in the Sakaguchi phenomenon. Analysis of mathematical models of suppression by regulatory T cells suggested that the persistence and growth of the regulatory population is dependent on the autoreactive effector T cells they control, and that this dependency will increase the efficiency of suppressive function. This crosstalk between regulatory and effector cells can fully account for adoptive transfers of tolerance by CD4+CD25+ T cells, as well as for several other features of tolerance. In the following, we extend the discussion, considering more generally the problem of self-tolerance regulation and also the related issue of adaptation of the immune system to chronic antigen stimuli.

4.1. Tuning of activation thresholds and suppression by regulatory T cells

Tunable activation thresholds and suppression by regulatory T cells are not mutually exclusive mechanisms of self-tolerance. Already in their original proposal, Grossman and Paul [17] suggested that anergic cells, with higher activation thresholds, could render naive cells anergic. Suppression by anergic cells has been shown in vitro [35,51], albeit the results are controversial [27], and the suppressive mechanism is not defined.

What dynamic properties are expected if T cell anergy is induced and maintained both by interactions with APCs and by interactions with other anergic T cells? We earlier studied [33] a mathematical model in which T_R cells convert T_E cells into the regulatory phenotype, showing that it has the same properties of a model in which T_R cells receive a growth factor from T_E cells. What would tunable activation threshold bring in addition to this? Consider the dependency of the fraction α of T_E and T_R cells activated upon conjugation with APCs on the frequency of conjugations and tuning. Consider also the frequency of T cell-APC interactions at the stable steady states of the (R, E) system with fixed α . Essentially the phase plane of the system will be maintained if the parameters are such that conjugations with the APCs at the stable steady states are rare enough such that threshold tuning is not significant, i.e. α is practically constant; under these conditions, one would expect the extinction of both effector or regulatory T cells to be stable, instead of unstable as in Fig. 5C. However, if the parameters are such that the interactions with APCs are frequent enough to reduce the fraction of conjugated T_E and T_R cells that become activated

then the steady states may disappear. These additional complications of coupling suppression and anergy induction are nontrivial and require proper modeling. Grossman and Paul [18,19] have discussed these issues extensively and, although they do not provide explicit mathematical models, their “conceptual models” would be a good stepping-stone.

4.2. Adaptation in the immune system: cellular or populational?

Several lines of evidence indicate that the immune system shows adaptation to continuous antigenic stimuli. Typically, chronically stimulated T cell populations are shown to become unresponsive when tested as a bulk (e.g. [53,47]). Acquisition of this bulk unresponsiveness is often interpreted as the adaptation of individual cells by raising their activation thresholds, however, this interpretation is not unique. Indeed, Grossman and colleagues [17,19] have argued that adaptation could happen at the level of the population as well as at the level of the individual cell signaling machinery. The mathematical analysis described here well illustrates this argument, since the (R, E) models show adaptation of the populations to chronic stimuli much in the same way as the kinase and phosphatase in the individual cell model. Thus, in the (R, E) models, when the system is in the tolerance state (be it within the bistable or the globally stable regimes), a sudden increase in antigen-bearing APCs to a new set point will trigger a transient response corresponding to the orbit of the system attaining the new steady state. This will be characterised by a transient expansion of T_E cells that will be eventually controlled by T_R cells. In the Leon et al. model [31], within the bistability regime, a fast increase of APC density may force a tolerance state to switch to an autoimmunity state.

Given the above considerations, the question is to what extent the adaptation scored as acquisition of bulk T-cell unresponsiveness happens at the level of single cell signaling or at the population level? Answering this question experimentally is not straightforward. For example, to experimentally rule out that an interaction between T cells regulates a response requires the use of limiting dilution analysis or single cell analysis, where the interactions are greatly disfavored or simply prevented [11]. Only if the frequency of responder T cells is not affected by diluting away T cell interactions can one definitively conclude that there was single cell adaptation, and eventually quantify the extent of induction of single cell unresponsiveness. These assays are, however, rarely performed in assessing adaptation of bulk T cell responses, which prevents an unequivocal interpretation of the results.

In this article we used two simple models to gain insight into the tolerance in the Sakaguchi phenomenon. Can these simple models also be used to gain insight into the mechanism underlying the acquisition of bulk T cell unresponsiveness? We believe so. As we have seen, the T cell-density dependencies of unresponsiveness by suppression and by TAT-dependent single cell anergy are opposite to each other. Suppression increases as the density of T_R cells per APC increases, and bulk T cell responsiveness (i.e. the response of a mixture of T_R and T_E cells) should decrease accordingly. In contrast, the average activation threshold, in our model the activity of the inhibitory phosphatase, is tuned down when the ratio of T cells per APC increases (Fig. 3B), and therefore bulk T cell responsiveness should increase accordingly. This offers a sort of “rule of thumb” for assessing what might be the predominant mechanism of adaptation in a given experimental setting in which the bulk responsiveness can be measured as a function of T-cell density per APC.

4.3. How can efficient responses to foreign antigens and robust self-tolerance coexist in the immune system?

The most important question about any self-tolerance mechanism is: how can efficient immune responses to foreign pathogens be mounted, while the immune system remains robustly self-tolerant?

The solution to this puzzle under the general TAT framework is that immune responses will be mounted to any antigen, self or foreign, whose presentation on the APCs increases suddenly [18]. Using a mathematical model, Scherer et al. [44] have shown that raising the activation thresholds of autoreactive T cells in the thymus, as posited before by Grossman and Singer [20], would be a more efficient way of ensuring efficient self-nonself discrimination than classical deletional mechanisms. Based on a TAT model, featuring also a TCR-dependent kinase–phosphatase cycle, Vand De Berg and Rand [56] have argued that tuning would render T cells uniform across repertoire and space, in terms of their capacity to respond to foreign antigens, and that pre-tuning in thymus would facilitate tolerance to self-antigens in the periphery. We reached a similar conclusion using our Monte-Carlo simulations [49]. Furthermore, the individual cell responses to foreign antigens would be facilitated and more sustained if the increase in the magnitude of the stimulus per APC (σ) is not concomitant with a large increase of stimulatory APCs. Hence, an increase in APCs, which is often associated with infections, will increase the frequency of conjugation events and therefore facilitates adaptation. This facilitation of adaptation might be counteracted in vivo by the fact that once the T cells are activated they lower their thresholds of activation, and perhaps become more resistant to tuning [19,23].

Regarding tolerance mediated by regulatory T cells, one of the aspects of the question above is that foreign antigens are always co-presented with self-antigens, and therefore autoreactive T cells could prevent immune responses. Based on simulation results, we have argued that immune responses can be efficiently elicited to those foreign antigens that displace sufficient self-antigens from the APCs and/or that are presented concomitantly with a marked increase in APCs. Another perhaps complementary solution, suggested by the typical bifurcation diagrams of the (R, E) models (Fig. 5B and C), is that the repertoire of regulatory T cells would be strongly biased towards self-antigens. Consider a scenario in which most T cell clones in circulation recognize too few APCs to sustain regulatory T cells. This is not unlikely given the fact that thymic deletion eliminates those T cells that would respond strongly to ubiquitous antigens. These T cell clones will contain only T_E cells but they would not cause autoimmunity because their expansion is limited by too few available APCs. Rarer T cell clones will recognize enough APCs such that they could expand to very high numbers, and thus could cause autoimmunity. In this case, however, APC-density is sufficient to sustain T_R cells, and thus clonal expansion is controlled. Although within this bistability regimen the autoreactive clones can reach either autoimmunity or tolerance, robust tolerance to these antigens will follow if the thymus exports enough T_R cells to ensure that any (R, E) population will be seeded within the basin of attraction of the state of T_R – T_E coexistence [32]. In this scenario, our model predicts that the T cell repertoire can be divided into two sets of lymphocyte clones: a larger, more diverse set of small clones containing only T_E cells, and a less diverse set of small clones, containing both T_E and T_R cells. In the first set, clonal sizes are determined only by APC availability, while in the second set clonal sizes are determined by suppression mediated by regulatory T cells. The dynamics of the first set would be that of the competition system modeled by De Boer and Perelson [9,10]. The dynamics of the second set would be that of the system studied by Leon et al. [32,33]. In this scenario, immune responses driven mainly by an increase in APCs would be obtained from the first set of clones, while tolerance to self would be ensured by the second set of clones. The plausibility of this

scenario depends critically on TCR crossreactivity and copresentation of peptides on the same APCs: whether a foreign antigen will elicit an immune response will depend on how many clones from the first and the second set will recognize peptides on the same APCs [32]. The constraints on repertoire size and crossreactivity/copresentation necessary for efficient self-nonself discrimination have been studied under the assumption that tolerance is mediated by clonal deletion ([8,12] and reference therein) and more recently by tuning [44,56]. The scenario we propose offers also another type of constraint on repertoire sizes and crossreactivity that could be amenable to similar modeling studies.

5. Concluding remarks

Hitherto the mechanisms of self-tolerance are essentially unresolved. We have used mathematical models to gain insights into these mechanisms. The models were designed as simple as possible in order to allow a better understanding of their knots and bolts. Therefore, while evidently unrealistic, such models may provide clues on how to make them more realistic. Despite their conceptual simplicity the models are highly nonlinear, requiring nontrivial analysis. Model analysis was based mainly on simple phase-plane and bifurcation analysis, which can be related to biology in straightforward, generic ways. We bootstrap the lack of analytic solutions through quasi-steady state approximations, graphical representations, and numerical solutions. Our conclusions are grounded on worked examples from other fields notably from statistical mechanics, and population dynamics. More than discussing the details of the mathematical derivations, which can be found in other publications, we have discussed and compared the assumptions and interpretations of different models. We believe that such continued critical discussion is instrumental in uncovering the basic rules of the immunological game, by producing more realistic models.

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Appendix A. Derivation of the quasi-steady state model of a single T-cell population

The diagram in Fig. 1A can be translated into the following two differential equations and one conservation equation:

$$\frac{dT_F}{dt} = (1 + \alpha)dC - cT_F A_F - \delta \cdot T_F, \quad (\text{A.1})$$

$$\frac{dC}{dt} = cT_F A_F - dC, \quad (\text{A.2})$$

$$A = A_F + C, \quad (\text{A.3})$$

where T_F is the density of free T cells, T_C is the density of APC-T-cell conjugates, A_F is the density of free APCs and A is the total density of APCs. The parameters are the rate constant of conjugate formation c , the rate constant of conjugate dissociation d , and the death rate constant δ . α is the probability that a T-cell is activated following activation. It depends on the internal state of the T-cell and it is defined according to Eq. (10) in the main text, which uses the stationary probability density function of the phosphatase activity in conjugated cells derived in Appendix B.

We are interested in following the total density of T cells in time denoted T :

$$T = T_F + C. \quad (\text{A.4})$$

One practical reason to do this is that with the available experimental techniques it is very difficult to count free and conjugated T cells in vivo. Instead experimentalists isolated the mixture of T cells (conjugated or free) and counted them.

Taking the derivative of both sides of Eq. (A.4) we obtain:

$$\frac{dT}{dt} = \frac{dT_F}{dt} + \frac{dC}{dt} = d\alpha C - \delta T_F = d\alpha C - \delta(T - C) \quad (\text{A.5})$$

which is to Eq. (1) in the main text.

Assuming that the conjugates are in quasi-steady state we have:

$$\frac{dC}{dt} = cT_F A_F - dC = 0. \quad (\text{A.6})$$

Substituting T_F and A_F by their expression in terms of A , T and T_C we obtain a second-order equation

$$c(T - C)(A - C) - dC = 0. \quad (\text{A.7})$$

Solving it we obtain two solutions, a negative and a positive. Only the positive solution is physically meaningful and thus was considered and corresponds to Eq. (2) in the main text.

For phase-space and bifurcation analyses of this one-dimensional model we used the software Mathematica. The steady states were calculated numerically for each combination of parameters using the FindRoot routine of Mathematica, which implements the Newton method. The stability of each of these solutions was determined by linear stability analysis.

Appendix B. Derivation of the stationary distribution of phosphatase activity in a population of T cells

The dynamics of probability density functions (PDFs) of the phosphatase activity in the subpopulations of conjugated and free T cells, respectively, ρ_C and ρ_F , are described by the following set of first-order partial differential equations:

$$\frac{\partial \rho_C}{\partial t} + \frac{\partial}{\partial P}(P_C \rho_C) = -d\rho_C + c_E \rho_F, \quad (\text{B.1})$$

$$\frac{\partial \rho_F}{\partial t} + \frac{\partial}{\partial P}(P_F \rho_F) = d\rho_C - c_E \rho_F, \quad (\text{B.2})$$

where P_C and P_F are the functions governing the dynamics of the phosphatase in the conjugated and free regimes (i.e. the right-hand side of Eq. (4) with $\sigma > 0$ and $\sigma = 0$, respectively):

$$P_C = r_P(P_0(1 + \sigma) - P), \tag{B.3}$$

$$P_F = r_F(P_0 - P) \tag{B.4}$$

and $c_E = cA_F = c(A - C)$ and d are the per T cell transition rates from the free to the conjugated state and from the conjugated to the free state, respectively.

In search for the steady state solutions we make $\partial P_C / \partial t = 0$, $\partial P_F / \partial t = 0$ and obtain the following set of ordinary differential equations:

$$\frac{\partial}{\partial P}(P_C \rho_C) = -d\rho_C + c_E \rho_F, \tag{B.5}$$

$$\frac{\partial}{\partial P}(P_F \rho_F) = d\rho_C - c_E \rho_F. \tag{B.6}$$

Noticing that the right-hand sides of these two equations are symmetrical we can add them obtaining the following conservation:

$$\frac{\partial}{\partial P}(P_C \rho_C + P_F \rho_F) = 0, \tag{B.7}$$

which upon integration leads to:

$$P_C \rho_C + P_F \rho_F = K. \tag{B.8}$$

Because ρ_C and ρ_F are PDFs, which cannot be negative, there must be at least one value P_1 such that $\rho_C(P_1) = \rho_F(P_1) = 0$. Therefore we have $K = 0$ which leads to the following relation:

$$P_F \rho_F = -P_C \rho_C. \tag{B.9}$$

Solving this equation for ρ_F and substituting in Eq. (B.2) we obtain the following ordinary differential equation:

$$\frac{\partial \rho_C}{\partial P} = \left(-\frac{d}{P_C} - \frac{c_E}{P_F} - \frac{\partial P_C}{\partial P} \right) \rho_C. \tag{B.10}$$

The solution of this equation is

$$\rho_C = N e^{\int -d/P_C - c_E/P_F - (\partial P_C / \partial P) / P_C dP}. \tag{B.11}$$

Given the definitions of P_F and P_C according to Eqs. (B.3) and (B.4) the integrals are:

$$\int -\frac{d}{P_C} dP = \log |P_0(1 + \sigma) - P|^{\frac{d}{r_P}}, \tag{B.12}$$

$$-\int \frac{c_E}{P_F} dP = \log |P_0 - P|^{\frac{c_E}{r_F}}, \tag{B.13}$$

$$\int -\frac{\partial P_C}{\partial P} \frac{1}{P_C} dP = \log |P_0(1 + \sigma) - P|^{-1}. \quad (\text{B.14})$$

Replacing these integrals in Eq. (B.11) we get the following equation that corresponds to Eq. (9) in the main text.

$$\rho_C = \begin{cases} N|P_0(1 + \sigma) - P|^{(d/r_P)-1}|P_0 - P|^{c_E/r_P}, & P_0 \leq P \leq P_0(1 + \sigma), \\ 0 & \text{otherwise.} \end{cases} \quad (\text{B.15})$$

The solution is branched because at the steady state the values of P are always contained in the interval $[P_0, P_0(1 + s)]$, whose extremes are the steady state values of P predicted according to Eq. (4) in the main text if T cells would be either always free or always conjugated, respectively.

Appendix C. Derivation of quasi-steady state model of T-cell population containing T_R and T_E cells

The diagram in Fig. 4 can be translated into the following set of differential equations and one conservation equation:

$$\frac{dR_F}{dt} = 2sR_A E_A + rR_A + d(1 - \alpha)R_C - cR_F A_F - \delta \cdot R_F, \quad (\text{C.1})$$

$$\frac{dE_F}{dt} = sR_A E_A + 2pE_A + rE_A + d(1 - \alpha)E_C - cE_F A_F - \delta \cdot E_F, \quad (\text{C.2})$$

$$\frac{dR_C}{dt} = -dR_C + cR_F A_F, \quad (\text{C.3})$$

$$\frac{dE_C}{dt} = -dE_C + cE_F A_F, \quad (\text{C.4})$$

$$\frac{dR_A}{dt} = d\alpha R_C - sR_A E_A - rR_A, \quad (\text{C.5})$$

$$\frac{dE_A}{dt} = d\alpha E_C - sR_A E_A - pE_A - rE_A, \quad (\text{C.6})$$

$$A = A_F + R_C + E_C, \quad (\text{C.7})$$

where R_F (or E_F) is the density of free T_R (or T_E) cells, R_C (E_C) is the density of conjugated T_R (T_E) cells, R_A (E_A) is the density of activated T_R (T_E) cells, A_F is the density of free APCs, and A is the total density of APCs. The parameters are the rate constant of conjugate formation c , the rate constant of conjugate dissociation d , the constant rate of reversion of the activated state to the resting state r , the constant rate of division of activated T_E cells that give rise to two resting T_E cells p , the constant rate of suppression s which leads to the reversion of activated T_E cells to the resting state without division but will lead to division of activated T_R cells into two resting T_R cells, and the death rate constant δ . α is the probability that a T-cell is activated following activation. Biologically, it is the same α as in the model in Appendix A. In the model of tuning we made it dependent on the internal state of the cell and calculated it according to the PDF of P , but here it is a simple constant.

As before (Appendix A), we are interested in the dynamics of the total densities of T_R and T_E , denoted R and E , respectively. The respective derivatives are:

$$\frac{dR}{dt} = \frac{dR_F}{dt} + \frac{dR_C}{dt} + \frac{dR_A}{dt} = sE_A R_A - \delta R_F, \tag{C.8}$$

$$\frac{dE}{dt} = \frac{dE_F}{dt} + \frac{dE_C}{dt} + \frac{dE_A}{dt} = pE_A - \delta E_F. \tag{C.9}$$

For maximum simplicity, we assume that the densities of conjugated and activated T cells are negligible when compared to the total densities at any time point. Thus, we have

$$R = R_F + R_C + R_A \approx R_F, \quad E = E_F + E_C + E_A \approx E_F. \tag{C.10}$$

These approximations (C.10) are valid as long as $c \ll d$ and $\alpha d \leq r$, which ensure that at equilibrium the density of conjugated cells will be much smaller than the density of free cells, and the density of activated cells is at maximum identical to the density of conjugated cells. Under these assumptions, Eqs. (C.8) and (C.9) become, respectively, Eqs. (11) and (12) in the main text.

We assume that the conjugate densities are in quasi-steady, obtaining the following expressions for R_C and E_C :

$$R_C = \frac{c}{d} A_F R_F, \quad E_C = \frac{c}{d} A_F E_F. \tag{C.11}$$

Substituting these expressions in the conservation equation APCs (Eq. (C.7)) and solving to A_F we obtain

$$A_F = \frac{A}{1 + \frac{c}{d} R_F + \frac{c}{d} E_F}. \tag{C.12}$$

Substituting back in Eq. (C.8) we obtain the following expressions for the conjugates of T_R and T_E cells:

$$R_C = \frac{AR_F}{\frac{d}{c} + R_F + E_F}, \quad E_C = \frac{AE_F}{\frac{d}{c} + R_F + E_F} \tag{C.13}$$

which leads to Eqs. (15) and (16) in the main text following the approximations in Eq. (C.10).

We assume also that the activated T cells are also in quasi-steady state. Setting $dR_A/dt = 0$ and solving Eq. (C.5) in order to R_A we obtain

$$R_A = \frac{\alpha d R_C}{r + s E_A}. \tag{C.14}$$

Setting $dE_A/dt = 0$ in Eq. (C.6) and substituting R_A according to Eq. (C.14) yields the following second-order equation in E_A :

$$0 = rd\alpha E_C + (sd\alpha E_C - s\alpha d R_C - r(p + r))E_A - s(p + r)(E_A)^2. \tag{C.15}$$

Only one of the two solutions is positive and thus biologically meaningful being presented as Eq. (14) in the main text.

For phase-space and bifurcation analyses of this two-dimensional (R, E) model we used the software Mathematica. Closed-form expressions were obtained for the nullclines, which are parabolic curves in

the plane (R, E) , albeit no closed expressions could be obtained for the nontrivial steady states. For each parameter set, the steady states were obtained numerically by finding all the intersections between the pairs of E and R nullclines, i.e. by finding the zeros of the subtraction of the two corresponding nullclines using the Newton method (implemented in the function FindRoot of the software). Linear stability analysis of each steady state was performed identifying stable and unstable states. The phase planes corresponding to a particular parameter set were drawn by plotting the nullclines and the stable and unstable steady states (represented as filled and empty circles, respectively) in the physically meaningful quadrant (both variables are null or positive). The bifurcation diagram as a function of parameter A was obtained by plotting the sum of the state variables $(R + E)$ in each physically meaningful steady state. Stable states are represented with continuous lines and unstable states with dashed lines.

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