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How T-cells use large deviations to recognize foreign antigens

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Abstract A stochastic model for the activation of T-cells is analysed. T-cells are part of the immune system and recognize foreign antigens against a background of the body's own molecules. The model under consideration is a slight generalization of a model introduced by Van den Berg, Rand and Burroughs in 2001 [18], and is capable of explaining how this recognition works on the basis of rare stochastic events. With the help of a refined large deviation theorem and numerical evaluation it is shown that, for a wide range of parameters, T-cells can distinguish reliably between foreign antigens and self-antigens.

Keywords immune system · T-cells · antigen-presenting cells · foreign versus self · kinetics of stimulation · large deviations · activation curves

Mathematics Subject Classification (2000) MSC 60F10 · MSC 92C37

1 Introduction

The mammalian immune system relies critically on so-called T-cells, which recognize foreign antigens and trigger an immune response. The word "antigen" is derived from *anti*body *gen*erating (indicating that antigens are molecules that can elicit an immune reaction).

Each individual is supplied with a large repertoire of different types of T-cells (each defined by the special type of T-cell receptor exposed at its surface), and every type recognizes a certain repertoire of antigens. This recognition, in turn, starts a signalling cascade, which induces an immune response that finally leads to the elimination of the antigen (Janeway et al. [9]).

The T-cell repertoire of an organism must, on the one hand, recognize *foreign* antigens in a reliable way; on the other hand, it must *not* respond to the body's *own* antigens, since this would elicit dangerous auto-immune reactions. How does this "self-nonself distinction" work?

This basic question of immunobiology has remained unanswered for a very long time. One fundamental difficulty lies in the fact that foreign antigens and self-antigens are very similar in nature. Van den Berg, Rand and Burroughs [18] (henceforth referred to as BRB) addressed this difficulty by modelling the *probabilistic* nature of the interactions between T-cell receptors and the antigens presented on the surface of so-called antigen-presenting cells (APCs). A T-cell (with many copies of its given receptor) encounters these APCs (which carry a random mixture of antigens). By modelling these encounters as random events,

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and taking the interaction kinetics between T-cell receptors and antigens into account, BRB have shown that the T-cell repertoire can distinguish reliably between APCs that carry foreign antigens and those that do not.

In order to validate the results of BRB, we reconsider their model and refine its analysis. In mathematical terms, the model boils down to computing the distribution of a large sum of independent but not identically distributed random variables. Since a T-cell response is a rare event (for a randomly chosen encounter), the tail of the distribution is relevant – a situation that requires the use of *large deviation theory*. Specifically, we need the so-called *exact asymptotics*, as provided by the Bahadur-Rao theorem and a generalization proved by Chaganty and Sethuraman [2]. With the help of this theorem, we find substantially elevated tail probabilities for the case where a foreign antigen is present in a fairly high copy number, relative to the self-background. Abundance of the foreign antigen is biologically realistic, since pathogens multiply within the body and swamp it with their antigens before an immune response is started. Furthermore, the requirement of a high copy number can be relaxed in a refined version of the model that includes a biological mechanism known as negative selection (see below).

The BRB paper [18] appeared in a biological context (with lots of immunological detail not easily accessible to the non-specialist) and therefore put little emphasis on mathematical detail. The aim of the present article is threefold. Firstly, we will make this fascinating piece of theoretical biology available to a more mathematical readership. To this end, we will streamline the modelling by formulating a set of explicit assumptions. Secondly, we will put the analysis on a solid mathematical basis by stating and applying the necessary large deviation result. This result holds under rather general conditions and therefore opens up interesting perspectives for further research. Thirdly, we will put forward and analyse numerically an extension of the BRB-model obtained by replacing the constant copy numbers of antigens on APCs by random variables, which is biologically more realistic.

The remainder of this article (which builds on the thesis of Zint [27], where many more details may be found) is organized as follows. Section 2 explains the immunological problem in a nutshell. Section 3 presents the mathematical model in its generalized form, with the emphasis on making individual modelling steps and assumptions transparent. Section 4 is devoted to large deviations, and provides the main theorem required for our analysis. On this basis, approximations are derived in Section 5, and applied to the biological model to demonstrate its recognition ability. Section 6, finally, summarizes and discusses the results, the possible extensions, as well as the limitations of the model.

2 T-cells and antigen recognition in a nutshell

The object of immunobiology is the body's own defence against pathogens like bacteria, viruses or fungi. One distinguishes between unspecific and specific defence mechanisms. The latter form the so-called immune system, which specifically reacts to intruders. In this reaction, the T-cells play an important role, which we will now briefly describe; for more details, see the textbook by Janeway et al. [9].

T-cells. T-cells are produced in the bone marrow and subsequently migrate to the thymus, where they mature (see below). On leaving the thymus, each T-cell is characterized by a specific type of T-cell receptor (TCR), which is displayed in many *identical* copies on the surface of the particular T-cell (see Fig. 1). These TCRs play an important part in the recognition of intruders (see below). It is important to note that all TCRs on one T-cell are of the same type. However, a large number (roughly 10^7; see Arstila et al. [1]) of different receptors, and hence different T-cell types, are present in an individual.

Antigen-presenting cells. The partners of the T-cells are the antigen-presenting cells (APCs), each producing so-called MHC molecules of different types (the set of these different types is the same for all the APCs of a given individual). An APC absorbs antigens from its vicinity and breaks them down. In the cell the emerging fragments, so-called peptides (short sequences of amino acids), are bound to the MHC molecules. The resulting complexes, composed of an MHC molecule and a peptide (abbreviated by pMHC), are displayed on the surface of the cell (the MHC molecules serve as "carriers" or "anchors" to the cell surface). Since most of the peptides in the vicinity of an APC are the body's own peptides, every APC displays a large variety of different types of self-peptides and, possibly, one (or a small number of)

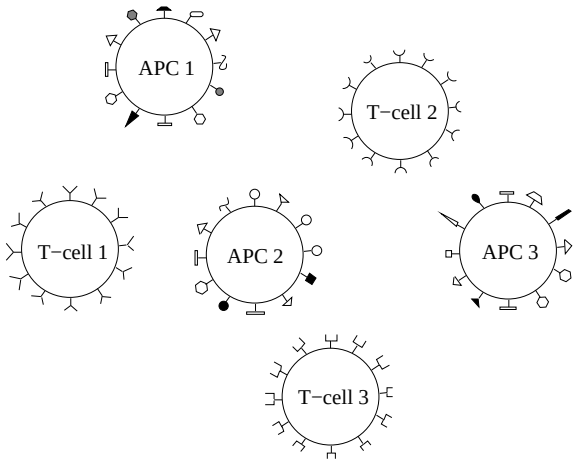


Fig. 1 A sample of different T-cells and APCs

foreign types. The various types of peptides occur in various copy numbers, as will be detailed below. For the moment, we merely note that foreign peptides are often present at elevated copy numbers. As noted above, this is because pathogens multiply within the body and flood it with their antigens, before an immune response is initiated.

Interactions between T-cells and APCs. The presentation of peptides on the surface of the APCs is of great importance for the immune system, because T-cells will only be activated when they recognize foreign peptides on the surface of an APC. The contact between a T-cell and an APC is established by a temporary bond between the cells, through which a so-called immunological synapse (see Fig. 2) is formed, in which the TCRs and the pMHCs interact with each other. If a T-cell recognizes a foreign peptide through its receptors, then it is activated to reproduce, and the resulting clones of T-cells will initiate an immune reaction against the intruder.

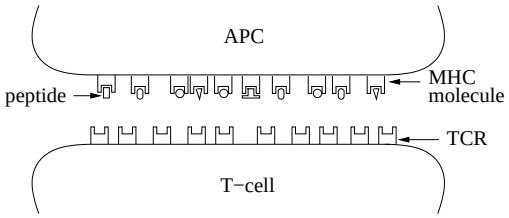


Fig. 2 An immunological synapse

Maturation. During the maturation of a T-cell, several processes take place in the thymus. Initially, the T-cell starts to display the TCRs on its surface. After this, two selective processes take place. During positive selection, those T-cells that hardly interact with the MHC molecules of the individual are removed. Furthermore, negative selection causes the removal of those T-cells that react too strongly to self-peptides. Thus, both useless and dangerous T-cells are removed.

Problem. Self-peptides and foreign peptides cannot differ from each other by nature. After all, even tissues of a different individual of the same species are recognized as foreign (this is the basic problem of transplantation). However, the activation of a T-cell occurs only when it recognizes a foreign peptide. Therefore the question comes up how the T-cells can distinguish between self and non-self. At first sight, the task seems hopeless, since there are vastly more different peptides (roughly 10^{13}; see Mason [12])

than TCRs (roughly 10^7, as noted earlier), which makes specific recognition (where one TCR recognizes exactly one pMHC) impossible; this is known as the Mason paradox. Fortunately, there is an answer to this question.

3 The model

We present here a slightly generalized version of the model originally proposed in BRB [18]. To this end, we recapitulate the modelling ideas and idealizations, and summarize them as assumptions (A1)–(A7) below.

Consider the immunological synapse between a T-cell and an APC (see Fig. 2). The activation of the T-cell is conceived as follows. If a contact between a TCR and a pMHC lasts longer than a certain time period, t^*, then the T-cell will receive a stimulus. The T-cell adds up the stimulation rates of all its receptors. If this sum exceeds a threshold, g_{act}, then the T-cell will be activated. This model relies on several hypotheses, which are known as kinetic proofreading ([3],[14]), serial triggering ([16],[17]) and counting of stimulated TCRs ([15],[24]).

3.1 Kinetics of stimulation

Let R_i be an unbound TCR of type i, M_j an unbound pMHC of type j, and C_{ij} a complex composed of R_i and M_j, where $i, j \in \mathbb{N}$. For every such pair, the binding and unbinding may, in chemical shorthand notation, be symbolized as

$$\mathrm{R}_i + \mathrm{M}_j \underset{\rho_{ij}}{\overset{\lambda_{ij}}{\rightleftharpoons}} \mathrm{C}_{ij} \ , \tag{1}$$

where λ_{ij} and ρ_{ij} are the association and dissociation rates, respectively. An encounter (to be used synonymously with an immunological synapse) between a T-cell and an APC with its mixture of pMHCs is therefore characterized by the type i of the T-cell, the types j of pMHCs at hand and the associated surface densities z_j, as well as the rates λ_{ij} and ρ_{ij} (which will be considered fixed for the purpose of this subsection). If the spatial structure in the immunological synapse is ignored, then the corresponding kinetics in the synapse is described by the deterministic law of mass action, i.e., for the given i,

$$\begin{aligned}
\frac{\mathrm{d}}{\mathrm{d}t} c_{ij}(t) &= \lambda_{ij} r_i(t) m_j(t) - \rho_{ij} c_{ij}(t) \ , & c_{ij}(0) &= 0 \ , \quad \forall j \ , \\
\frac{\mathrm{d}}{\mathrm{d}t} m_j(t) &= -\lambda_{ij} r_i(t) m_j(t) + \rho_{ij} c_{ij}(t) \ , & m_j(0) &= z_j \ , \quad \forall j \ , \\
\frac{\mathrm{d}}{\mathrm{d}t} r_i(t) &= -\sum_j \lambda_{ij} r_i(t) m_j(t) + \sum_j \rho_{ij} c_{ij}(t) \ , & r_i(0) &= r \ ,
\end{aligned} \tag{2}$$

where $r_i(t)$, $m_j(t)$ and $c_{ij}(t)$ are the surface densities of R_i, M_j and C_{ij}, respectively, at time t (and we note that only finitely many z_j's are non-zero). It is easily verified that the solution of (2), for the given i, satisfies the conservation laws

$$r_i(t) + \sum_j c_{ij}(t) = r \qquad \text{and} \qquad m_j(t) + c_{ij}(t) = z_j \quad \forall j \ . \tag{3}$$

Note that this deterministic approach, with its surface densities varying over the reals rather than a finite set, is justified when the numbers of all the involved molecules are large enough (see e.g. Ethier and Kurtz [6, Chapter 11, Theorem 2.1]). This standard approach to reaction kinetics is also used for the binding kinetics in the immunological synapse (see e.g. BRB [19, Eq. (A.5)], which describes the equilibrium of this model). It should be noted, however, that some of the antigen types may be fairly rare in the situation at hand, so that the deterministic approach may be somewhat crude. But it will become clear later on that we actually do not depend on details of the binding kinetics.

[Remark: In the probabilistic approach in the next subsection, we will use the symbol z_j for the copy number of type j pMHC rather than its surface density, since they differ by an (irrelevant) normalization factor only.]

Equilibrium. The bond between a TCR and a pMHC consists of two parts: contacts between the TCR and the MHC molecule, and contacts between the TCR and the peptide. According to Wu et al. [26], the former specify mainly the association rate and the latter mainly the dissociation rate. We consider mature T-cells, which implies that they have been positively selected, i.e., each T-cell binds one type of the MHC molecules of the individual very well. Thus, we idealize λ_{ij} as very large ($\gg 1/(r\,t^*)$) for pMHCs containing this type of MHC molecule, and zero otherwise. For every i, we therefore restrict j in (2) to the set

$$\mathcal{P}_i = \{j\colon\ \lambda_{ij} \gg 1/(r\,t^*)\}\ , \tag{4}$$

since only these pMHCs contribute significantly to the stimulation rate. As a consequence, we may assume that the reaction is in equilibrium, because this is reached on a time scale that is short relative to the time scale of activation. Therefore, in view of (2), for every i we have

$$\hat{c}_{ij} = \frac{\lambda_{ij}}{\rho_{ij}}\,\hat{r}_i\hat{m}_j \qquad \forall\, j \in \mathcal{P}_i\ , \tag{5}$$

where $\hat{r}_i$, $\hat{m}_j$ and $\hat{c}_{ij}$ denote the equilibrium quantities. Combining (3) for these quantities with (5), we get an equation for $\hat{c}_{ij}$ that can be solved to give the implicit system of equations

$$\hat{c}_{ij} = z_j\,\frac{r - \left(\sum_{k\in\mathcal{P}_i}\hat{c}_{ik}\right)}{r - \left(\sum_{k\in\mathcal{P}_i}\hat{c}_{ik}\right) + \rho_{ij}/\lambda_{ij}}$$

(cf. BRB [19, Appendix A.1]). Assuming further that the concentration of TCRs is not limiting (i.e., $r > \sum_{j\in\mathcal{P}_i} z_j$ – this is the so-called serial triggering regime) and that the relevant dissociation rates are very small (i.e., $\rho_{ij}/\lambda_{ij} \ll r$ for all $j \in \mathcal{P}_i$), we are led to the idealization

$$\hat{c}_{ij} = z_j\ . \tag{A1}$$

As will become clear later on, this assumption may actually be relaxed (see Section 6); we make it here for ease of exposition.

Stimulation rate. A given C_{ij} dissociates at rate ρ_{ij}. Therefore, T_{ij}, the duration of a contact between R_i and M_j, is exponentially distributed with mean $\tau_{ij} = 1/\rho_{ij}$. Hence, the probability of T_{ij} exceeding t^* is $\mathrm{e}^{-t^*/\tau_{ij}}$, which we refer to as the *stimulation probability* (of a C_{ij}). Note that, by the above together with (4) and (A1), an encounter between a T-cell and an APC is now characterized by i, $\mathcal{P}_i$ as well as the collections of z_j and τ_{ij} for all $j \in \mathcal{P}_i$ with $z_j > 0$.

The T-cell receives a stimulus every time a complex dissociates that has existed for at least time t^*. Therefore the average stimulation rate of type ij complexes is given by

$$\rho_{ij}\,\mathrm{P}(T_{ij} > t^*) = w(\tau_{ij}) \quad \text{with} \quad w(\tau) = \tfrac{1}{\tau}\mathrm{e}^{\frac{-t^*}{\tau}}\ . \tag{A2}$$

In Fig. 3, $w(\tau)$ is plotted as a function of τ. This curve can be interpreted as follows.

1. If $\tau \ll t^*$, then the complex will typically dissociate before stimulation.
2. If $\tau \gg t^*$, then the TCR and the pMHC will typically be associated for a long time. Therefore the T-cell will get a stimulus through practically every binding event, but the pMHC keeps the receptor occupied for a long time, so only few stimuli are expected per time unit.

By (A1) and (A2), we finally get the total stimulation rate for a conjunction of a T-cell of type i and a particular APC:

$$g_i = \textstyle\sum_{j\in\mathcal{P}_i} z_j w(\tau_{ij})\ . \tag{A3}$$

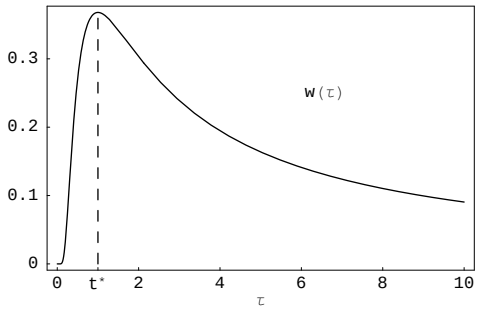


Fig. 3 Average stimulation rate of an individual complex type as a function of the average waiting time

3.2 A probabilistic approach

So far, $\mathcal{P}_i$, z_j and τ_{ij} are considered to be given quantities for all $i, j \in \mathbb{N}$. Indeed, z_j and τ_{ij} may be determined experimentally for a given i, j pair (cf. [8],[13]). However, owing to the diversity of complexes C_{ij} and mixtures of peptides presented on the APCs, it is not possible to specify all these quantities individually. Therefore, in order to derive the overall probability of T-cell activation, a probabilistic approach is required.

Presentation of antigens. The genes of an organism can be classified as constitutive ones and inducible ones. The former encode proteins that are always present in every cell (e.g. proteins of the basic metabolism). In contrast, the latter encode proteins that only exist in some cells (like for example muscle proteins) and/or occur only temporarily, i.e., they are variable. Accordingly, the types of self-peptides on each APC may be partitioned into constitutive and variable, i.e., $\mathcal{P}_i = \mathcal{C}_i \cup \mathcal{V}_i$, where $\mathcal{C}_i \cap \mathcal{V}_i = \emptyset$, and we suppose that:

(A4) There are constant numbers n_c and n_v of constitutive and variable types of peptides, respectively, on each APC.

The constitutive types ($\mathcal{C}_i$) are the same on each APC, whereas there is a different sample of variable types ($\mathcal{V}_i$) on each APC. As a generalization of BRB [18] and Zint [27], we allow the copy numbers of the individual types within each class to vary. Therefore we suppose that:

(A5) The z_j are realizations of random variables denoted by Z_j. These random variables are independent and identically distributed (i.i.d.) within each of the two classes, and are referred to as $Z_j^{(c)}$ and $Z_j^{(v)}$.

[Remark: The i.i.d. assumption is made for simplicity; we do not model a particular biological mechanism here. Realistic models would be based on MHC loading fluctuations (cf. BRB [18, Appendix C]). They invariably induce dependencies, the treatment of which is beyond the scope of the present paper.]

Let us now add in foreign peptides, and make the simplifying assumption that only one type of foreign peptide is present on an APC, in z_f copies. In the following, the index set for the pMHCs is therefore the union of $\mathbb{N}$ and $\{f\}$.

The total stimulation rate with respect to a conjunction of a T-cell of type i and a randomly chosen APC is given by

$$G_i(z_f) = \left(\sum_{j \in \mathcal{C}_i} q Z_j^{(c)} w(\tau_{ij}) \right) + \left(\sum_{j \in \mathcal{V}_i} q Z_j^{(v)} w(\tau_{ij}) \right) + z_f w(\tau_{if}) \; .$$

Here, the factor $q = (n_M - z_f)/n_M$ ensures that adding the foreign peptides does not change the expected number $n_M = n_c \, \mathrm{E}(Z_1^{(c)}) + n_v \, \mathrm{E}(Z_1^{(v)})$ of MHC molecules on the surface of an APC, since also $q(n_c \, \mathrm{E}(Z_1^{(c)}) + n_v \, \mathrm{E}(Z_1^{(v)})) + z_f = n_M$.

Probability of activation. In line with the original model, we suppose that:

(A6) For all i and j, the τ_{ij} are realizations of i.i.d. random variables $\mathcal{T}_{ij}$ with mean $\bar{\tau}$ (see BRB [18] and Zint [27] for explanations).

In particular, this assumption means that no distinction between foreign and self is built into the interaction between receptors and antigens. This reflects the fact that there is no a-priori difference between the peptides.

Note that (A6) also implies that the $\mathcal{P}_i$ need not be specified and the index i may be suppressed, because we consider an arbitrary T-cell and the G_i are i.i.d. random variables. Biologically, this means that we choose a new T-cell type (as well as a new APC) for each encounter, and ignore further meetings of T-cells of the same type. As a consequence, the partitioning into constitutive and variable peptides has, at this stage, no effect except for the different abundances.

Altogether the total stimulation rate with respect to a conjunction of a randomly chosen T-cell and an APC is given by

$$G(z_f) = \left(\sum_{j=1}^{n_c} q Z_j^{(c)} W_j\right) + \left(\sum_{j=n_c+1}^{n_c+n_v} q Z_j^{(v)} W_j\right) + z_f W_{n_c+n_v+1}\ , \tag{6}$$

where $W_j = w(\mathcal{T}_j)$, and:

(A7) The probability of T-cell activation is $\mathrm{P}\left(G(z_f) \geq g_{\mathrm{act}}\right)$.

Specification of distributions and parameter values. For ease of exposition (and in line with the original model), $\mathcal{T}_{ij}$ is exponentially distributed and we have chosen $t^* = 1$, $\bar{\tau} = 0.04\, t^*$, $n_c = 50$ and $n_v = 1500$. As stated in BRB [18], the number of MHC molecules ranges between 10^4 and 10^6. Therefore we take $n_M = 10^5$. Furthermore, we use binomial distributions $\mathrm{Bin}_{m_c,p}$ and $\mathrm{Bin}_{m_v,p}$ for $Z_j^{(c)}$ and $Z_j^{(v)}$, for all j, with parameters $m_c = 1000$, $m_v = 100$ and $p = 0.5$, so that the means $\mathrm{E}(Z_1^{(c)}) = 500$ and $\mathrm{E}(Z_1^{(v)}) = 50$ correspond to the values $z_c/n_M = 0.005$ and $z_v/n_M = 0.0005$ in the BRB-model. (Apart from the expectation, the distribution is an ad-hoc choice.)

It should be mentioned that moderate changes of the values of the parameters (like for example n_c, n_v, $\mathrm{E}(Z_1^{(c)})$ and $\mathrm{E}(Z_1^{(v)})$) do not qualitatively alter the results. Furthermore, these values have been chosen on the grounds of experimental data (see BRB [18] and Zint [27]). The exponential distribution is less-well founded. To show that the qualitative behavior does not rely on this particular distribution, we will, as an alternative, use the log-normal distribution (with parameters $\mu = -3.3$ and $\sigma = 0.5$) in Section 5.2. This has a justification in terms of binding/unbinding kinetics (see BRB [18] and Zint [27]).

3.3 Distinction between foreign and self

For the immune system to work, two conditions are essential: (a) if a foreign antigen is present, then at least one T-cell will be activated; (b) there will be no activation when only self-antigens are present.

It is helpful to recast this into a hypothesis testing framework, in the following way. The immune system performs a test of the null hypothesis

$$H_0 :\ z_f = 0 \tag{7}$$

against the alternative hypothesis

$$H_A :\ z_f > 0\,. \tag{8}$$

The test is performed via N independent encounters between a T-cell and an APC. H_0 is then rejected (and H_A assumed) if at least one encounter leads to the event $\{G \geq g_{\mathrm{act}}\}$; otherwise, H_0 is retained. The type I error is therefore

$$\alpha = \mathrm{P}(H_A \text{ assumed} \mid H_0 \text{ true}) = 1 - \left(1 - \mathrm{P}\left(G(0) \geq g_{\mathrm{act}}\right)\right)^N,$$

and the type II error is

$$\beta = \mathrm{P}(H_0 \text{ assumed} \mid H_A \text{ true}) = \left(1 - \mathrm{P}\left(G(z_f) \geq g_{\mathrm{act}}\right)\right)^N\ . \tag{9}$$

Here, the underlying assumptions are (A1)–(A7), and $G(z_f)$ is the sum of random variables introduced in Equation (6), with constants n_c, n_v and parameter z_f. In particular, $G(0)$ denotes the total stimulation rate in the absence of foreign peptides. The parameter g_{act} can be fine-tuned by the cell; for more on activation threshold tuning, see Van den Berg and Rand [22].

Clearly, α is the probability of an autoimmune response, whereas β is the probability that a foreign antigen goes unnoticed. By (b) and (a) above, both α and β must be small for the self-nonself distinction to work.

Both $\mathrm{P}(G(0) \geq g_{\mathrm{act}})$ and $\mathrm{P}(G(z_f) \geq g_{\mathrm{act}})$ are rare events (since at most a tiny fraction of the T-cells reacts to a given APC). Therefore, α is close to 0 (close to 1) and β is close to 1 (close to 0) for N small (N very large). We have no good knowledge of the value of N (except that it is bounded above by the number of T-cell types). But it is clear that a necessary condition for distinction is that g_{act} can be chosen in such a way that, for physiologically realistic values of z_f,

$$\mathrm{P}(G(z_f) \geq g_{\mathrm{act}}) \gg \mathrm{P}(G(0) \geq g_{\mathrm{act}}). \tag{C1}$$

Consequently, there is a region of intermediate values of N for which both α and β are small. We thus need an analysis of the tiny probabilities $\mathrm{P}(G(0) \geq g_{\mathrm{act}})$ and $\mathrm{P}(G(z_f) \geq g_{\mathrm{act}})$. This requires large deviation theory and is the subject of Section 4.

4 Large deviations

For many families of random variables, *large deviation principles* (LDP's) are available to characterize their atypical behaviour. Here, we will be concerned with sums of random variables, i.e.,

$$S_n = \sum_{i=1}^{n} X_i,$$

where $(X_i)_{i\geq 1}$ is a sequence of independent (but *not* necessarily identically distributed) random variables (like those in Eq. (6)). An LDP characterizes the probability of a large deviation of S_n from its expectation; a *large deviation* is a deviation of order n (in contrast to a *normal deviation* of order $\sqrt{n}$, as covered by the central limit theorem). A basic result is Cramér's theorem, which says the following. For a sequence $(X_i)_{i\geq 1}$ of i.i.d. real-valued random variables whose moment-generating function $\phi(\vartheta) = \mathrm{E}(\exp(\vartheta X_1))$ is finite for all $\vartheta \in \mathbb{R}$, one has, for all $a > \mathrm{E}(X_1)$,

$$\lim_{n\to\infty} \frac{1}{n} \log \mathrm{P}(S_n \geq an) = -I(a), \tag{10}$$

where $I(a) = a\vartheta_a - \psi(\vartheta_a)$, $\psi(\vartheta) = \log \phi(\vartheta)$, and ϑ_a is the (unique) solution of $\psi'(\vartheta) = a$. That is, for large n, the probability that S_n is larger than an decays exponentially with n, with decay rate $I(a)$. The value ϑ_a is known as the *"tilting" parameter*. It is used for an exponential reweighting (or "tilting") of the distribution of the X_i (and hence of S_n) that inflates the right-hand tail of the distribution in such a way that the rare event $\{S_n \geq an\}$ turns into a typical one; this is a crucial step in the analysis. For a review of large deviation theory, see e.g. Den Hollander [4]; Cramér's theorem and its proof are found in Ch. I.3.

Note, however, that the knowledge of the exponential decay rate $I(a)$ alone does not suffice to provide meaningful leading-order estimates of the probabilities of the rare event itself. This is because (10) is compatible with $\mathrm{P}(S_n \geq an) = f(n) \exp\left(-nI(a)(1 + o(1))\right)$ for any prefactor $f(n) = \mathcal{O}(n^\alpha)$, with arbitrary α. More accurate information is obtained from so-called *exact asymptotics*. For the situation at hand, this is given by a refinement of Cramér's theorem due to Bahadur and Rao (cf. [5], Ch. 3.7). Namely, under the assumptions of Cramér's theorem and the additional requirement that the distribution of X_1 be non-lattice (which is always fulfilled if X_1 has a density), one has

$$\mathrm{P}(S_n \geq an) = \frac{1}{\sqrt{2\pi n}\sigma\vartheta_a} e^{-nI(a)}(1 + o(1)) \text{ as } n \to \infty \tag{11}$$

for all a that satisfy $\mathrm{E}(X_1) < a < \sup_\vartheta \psi(\vartheta)$. Here, $I(a)$ and ϑ_a are as above, and $\sigma^2 = \psi''(\vartheta_a)$ is the variance of $\frac{1}{n}S_n$ after "tilting" with the exponential parameter ϑ_a. (The condition $a < \sup_\vartheta \psi(\vartheta)$ ensures

that only those events $\{S_n \geq an\}$ are considered that are actually possible; the condition is void if X_1, and hence S_n/n, take values in all of $\mathbb{R}_{\geq 0}$.)

What we need to tackle our stimulation rates (6) is the generalization of (11) to situations in which the $(X_i)_{i\geq 1}$ are *not* identically distributed. Fortunately, a very general result is available, which does not even require independence. This is the result of Chaganty and Sethuraman [2], which plays a crucial role in our analysis, and which we will now formulate.

Let $(S_n)_{n\in\mathbb{N}}$ be a sequence of $\mathbb{R}$-valued random variables, with moment generating functions $\phi_n(\vartheta) = \mathrm{E}\left(\exp(\vartheta S_n)\right)$, $\vartheta \in \mathbb{R}$. Suppose that there exists a $\vartheta^* \in (0,\infty)$ such that

$$\sup_{n\in\mathbb{N}} \sup_{\vartheta\in B_{\vartheta^*}} \phi_n(\vartheta) < \infty \,, \tag{12}$$

where $B_{\vartheta^*} = \{\vartheta \in \mathbb{C}\colon\ |\vartheta| < \vartheta^*\}$. Define

$$\psi_n(\vartheta) = \frac{1}{n} \log \phi_n(\vartheta) \,, \tag{13}$$

and let $(a_n)_{n\in\mathbb{N}}$ be a bounded sequence in $\mathbb{R}$ such that for each n the equation

$$a_n = \psi_n'(\vartheta) \tag{14}$$

has a solution $\vartheta_n \in (0,\vartheta^{**})$ for some $\vartheta^{**} \in (0,\vartheta^*)$. This solution is unique by strict convexity of ψ_n. Define

$$\begin{aligned} \sigma_n^2 &= \psi_n''(\vartheta_n) \,, \\ I_n(a_n) &= a_n\vartheta_n - \psi_n(\vartheta_n) \,. \end{aligned} \tag{15}$$

Theorem 1 (Chaganty-Sethuraman [2]) *If* $\inf_{n\in\mathbb{N}} \sigma_n^2 > 0$, $\lim_{n\to\infty} \vartheta_n\sqrt{n} = \infty$ *and*

$$\lim_{n\to\infty} \sqrt{n} \sup_{\delta_1\leq|t|\leq\delta_2\vartheta_n} \left|\frac{\phi_n(\vartheta_n+it)}{\phi_n(\vartheta_n)}\right| = 0 \quad \forall\, 0<\delta_1<\delta_2<\infty \,, \tag{16}$$

then

$$\mathrm{P}\left(S_n \geq na_n\right) = \frac{\mathrm{e}^{-nI_n(a_n)}}{\vartheta_n\sigma_n\sqrt{2\pi n}}\,(1+o(1)) \qquad \textit{as } n\to\infty \,. \tag{17}$$

□

In analogy with the previous discussion, ϑ_n is the "tilting parameter" for the distribution of $\frac{1}{n}S_n$, σ_n^2 is the variance of the "tilted" $\frac{1}{n}S_n$, and $I_n(a_n)$ is the large deviation rate function. Let us further remark that, in principle, even finer error estimates (of Berry-Esséen type, cf. [7], Ch. XVI) can be obtained beyond the asymptotics in Theorem 1, but this becomes technically more involved.

5 Activation curves

In order to investigate whether condition (C1) can be fulfilled for physiologically realistic values of z_f, we consider the so-called activation curves, i.e., $1 - F_{z_f}(g_{\mathrm{act}})$ with F_{z_f} the distribution function of $G(z_f)$.

5.1 Simulation and approximation

We begin by deriving an approximation for the activation probability in condition (C1) based on Theorem 1. Consider a sequence of models defined by increasing numbers of constitutive and variable peptide types. Let

$$n = \begin{cases} n_c + n_v, & \text{if } z_f = 0, \\ n_c + n_v + 1, & \text{otherwise,} \end{cases}$$

and consider the limit $n \to \infty$ with $\lim_{n\to\infty} n_c/n_v = C_1 \in (0,\infty)$; note that the number of foreign peptides remains 1 throughout. Let S_n in Theorem 1 be

$$G_n(z_f) = \left(\sum_{j=1}^{n_c} q_n Z_j^{(c)} W_j\right) + \left(\sum_{j=n_c+1}^{n_c+n_v} q_n Z_j^{(v)} W_j\right) + z_f W_{n_c+n_v+1}$$

where

$$q_n = \frac{n_c\, m_c\, p + n_v\, m_v\, p - z_f}{n_c\, m_c\, p + n_v\, m_v\, p} \ ,$$

and let M_c, M_v and M be the moment generating functions of $Z_j^{(c)} W_j$, $Z_j^{(v)} W_j$ and W_j, respectively, i.e., for $\gamma \in \{c, v\}$,

$$M_\gamma(\vartheta) = \frac{1}{\overline{\tau}} \sum_{k=0}^{m_\gamma} \left(\int_0^\infty \exp\left(k\vartheta\, \frac{\exp(-t^*/\tau)}{\tau} - \frac{\tau}{\overline{\tau}} \right) \mathrm{d}\tau \right) \mathrm{Bin}_{m_\gamma,p}(k) \ , \tag{18}$$

and

$$M(\vartheta) = \frac{1}{\overline{\tau}} \int_0^\infty \exp\left(\vartheta\, \frac{\exp(-t^*/\tau)}{\tau} - \frac{\tau}{\overline{\tau}} \right) \mathrm{d}\tau \ . \tag{19}$$

Choose $a_n \equiv a$ and $g_{\mathrm{act}}(n) = an$. Let ϑ_n be the unique solution of

$$\begin{aligned} a = \quad & \frac{n_c}{n} q_n \left[\frac{\mathrm{d}}{\mathrm{d}\vartheta} \log M_c(\vartheta) \right] \Big|_{\vartheta = q_n \vartheta_n} \\ & + \frac{n_v}{n} q_n \left[\frac{\mathrm{d}}{\mathrm{d}\vartheta} \log M_v(\vartheta) \right] \Big|_{\vartheta = q_n \vartheta_n} + \quad \frac{1}{n} z_f \left[\frac{\mathrm{d}}{\mathrm{d}\vartheta} \log M(\vartheta) \right] \Big|_{\vartheta = z_f \vartheta_n} \ . \end{aligned} \tag{20}$$

We further define

$$\begin{aligned} \sigma_n^2 = \quad & \frac{n_c}{n} q_n^2 \left[\frac{\mathrm{d}^2}{\mathrm{d}\vartheta^2} \log M_c(\vartheta) \right] \Big|_{\vartheta = q_n \vartheta_n} \\ & + \frac{n_v}{n} q_n^2 \left[\frac{\mathrm{d}^2}{\mathrm{d}\vartheta^2} \log M_v(\vartheta) \right] \Big|_{\vartheta = q_n \vartheta_n} + \frac{1}{n} z_f^2 \left[\frac{\mathrm{d}^2}{\mathrm{d}\vartheta^2} \log M(\vartheta) \right] \Big|_{\vartheta = z_f \vartheta_n} \end{aligned} \tag{21}$$

and

$$I_n(a) = a\vartheta_n - \frac{n_c}{n} \log M_c(q_n \vartheta_n) - \frac{n_v}{n} \log M_v(q_n \vartheta_n) - \frac{1}{n} \log M(z_f \vartheta_n) \ . \tag{22}$$

Since we have only finitely many different types of random variables, all independent, it is straightforward to check

Lemma 1 *The conditions for Theorem 1 are satisfied.*

Proof The moment generating function $\phi_n(\vartheta)$ is given by

$$\phi_n(\vartheta) = \begin{cases} (M_c(\vartheta))^{n_c}\, (M_v(\vartheta))^{n_v} \ , & \text{if } z_f = 0, \\ (M_c(q_n\vartheta))^{n_c}\, (M_v(q_n\vartheta))^{n_v}\, M(z_f\vartheta), & \text{otherwise.} \end{cases}$$

Let z_f be fixed. If a is chosen such that $g_{\mathrm{act}}(n) > \mathrm{E}(G_n(0))$ and $g_{\mathrm{act}}(n) > \mathrm{E}(G_n(z_f))$ for all n (in which case the strict inequalities are in fact uniform in n), then $\lim_{n\to\infty} \vartheta_n = C_2 \in (0,\infty)$. Consequently, $\lim_{n\to\infty} \vartheta_n \sqrt{n} = \infty$, and also $\inf_{n\in\mathbb{N}} \sigma_n^2 > 0$. It thus remains to verify Condition (16). Let $F_c(x)$, $F_v(x)$ and $F(x)$ be the distribution functions of $Z_j^{(c)} W_j$, $Z_j^{(v)} W_j$ and W_j, respectively. Since

$$\nu_\gamma^{(n)}(t) = \frac{M_\gamma(q_n(\vartheta_n + it))}{M_\gamma(q_n\vartheta_n)} = \int_{\mathbb{R}} \exp(iq_n t x)\, \frac{\exp(q_n \vartheta_n x)}{M_\gamma(q_n\vartheta_n)}\, dF_\gamma(x), \qquad \gamma \in \{c, v\} \ ,$$

and

$$\nu^{(n)}(t) = \frac{M(z_f(\vartheta_n + it))}{M(z_f\vartheta_n)} = \int_{\mathbb{R}} \exp(iz_f t x)\, \frac{\exp(z_f \vartheta_n x)}{M(z_f\vartheta_n)}\, dF(x)$$

are characteristic functions of random variables that are not constant nor are lattice valued, and ϑ_n and q_n converge as $n \to \infty$, there exists an $\varepsilon > 0$ and an $n_0 < \infty$ such that, for all $t \neq 0$ and $n \geq n_0$, $|\nu_c^{(n)}(t)| \leq 1 - \varepsilon$, $|\nu_v^{(n)}(t)| \leq 1 - \varepsilon$ and $|\nu^{(n)}(t)| \leq 1 - \varepsilon$ (see Feller [7, Chapter XV.1, Lemma 4]). From this it follows that

$$\begin{aligned}
&\left|\frac{\phi_n(\vartheta_n + it)}{\phi_n(\vartheta_n)}\right| \\
&= \left|\frac{(M_c(q_n(\vartheta_n + it)))^{n_c}\,(M_v(q_n(\vartheta_n + it)))^{n_v}\,M(z_f(\vartheta_n + it))}{(M_c(q_n\vartheta_n))^{n_c}\,(M_v(q_n\vartheta_n))^{n_v}\,M(z_f\vartheta_n)}\right| \\
&= \left|\left(\nu_c^{(n)}(t)\right)^{n_c}\left(\nu_v^{(n)}(t)\right)^{n_v}\nu^{(n)}(t)\right| \\
&= o(1/\sqrt{n})
\end{aligned}$$

as $n \to \infty$ for all $t \neq 0$ (compare the argument leading to [7, Chapter XVI.6, Equation (6.6)]), which guarantees (16). □

In view of Lemma 1, we may approximate the probability of T-cell activation as

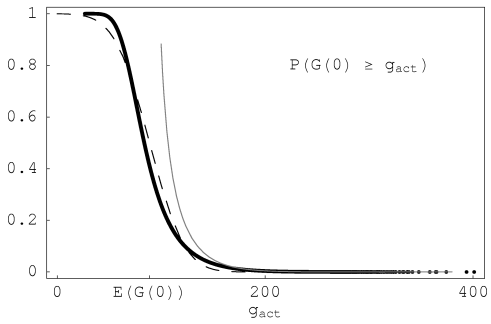
$$\mathrm{P}\left(G(z_f) \geq g_{\mathrm{act}}\right) \approx \frac{\mathrm{e}^{-nI_n(a)}}{\vartheta_n\sigma_n\sqrt{2\pi n}}\,, \tag{23}$$

where $G(z_f)$ is the original stimulation rate of (6), $g_{\mathrm{act}} = g_{\mathrm{act}}(n) = an$, and we assume that n is large enough for a good approximation. (Note that the threshold g_{act} is not known; but we do know that reactions of T-cells are rare events. Thus, we may assume that g_{act} is such that large deviations results are applicable, as will also be confirmed by our simulations below.) The expression in the right-hand side must be evaluated numerically (we used Mathematica® [25]), since already the moment generating functions in (18) and (19) are unavailable analytically, and this carries over to ϑ_n, σ_n^2, and $I_n(a)$ in (20)-(22).

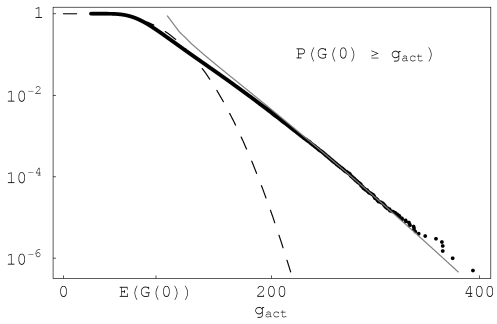
Let us consider the activation curve for two extreme cases, namely, the self-background ($z_f = 0$), and a very large number of foreign peptides ($z_f = 2500$). Fig. 4 shows the simulated curve in comparison to the normal approximation and the approximation in (23). As was to be expected, the normal approximation describes the central part well, whereas for the right tail (the relevant part of the distribution for the problem at hand) the large deviation approximation is appropriate. For $z_f = 0$, the latter describes the simulated distribution in an excellent way; for $z_f = 2500$, it still gives correct approximations beyond $g_{\mathrm{act}} = 550$, which is the region we are interested in (see the next subsection). An improved approximation of the entire curve is obtained in BRB [18] by combining the normal and the large deviation approximations, applying them to the self-peptides only, and performing a convolution with the single foreign one. We prefer the direct approach (23) here, because it makes the large deviation aspect more transparent, and because it generalizes easily to situations with more than one foreign peptide. In fact, rather than taking the limit in the way described above, we could as well consider a sequence of models with n_f different foreign peptides and let $n \to \infty$ such that n_c/n, n_v/n and n_f/n each tend to a constant; the approximation of our given finite system by (20)-(23) would remain unchanged.

5.2 Activation curves without negative selection

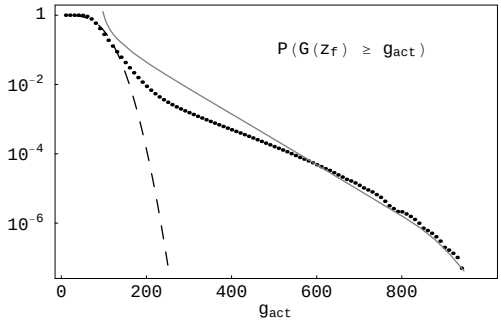
As we have seen in the last subsection, the approximation in (23) is suitable for the calculation of the activation curves for various values of z_f. Fig. 5 shows the curves as a function of g_{act} for exponentially (a) and log-normally (b) distributed $\mathcal{T}_{ij}$. The results in (a) and (b) are qualitatively the same. Namely, we observe that the curves for $z_f = 250$ and $z_f = 500$ (both $\leq \mathrm{E}(Z_1^{(c)}) = 500$) do not differ visibly from the curve for the self-background; but, for $z_f > 1000$ and $g_{\mathrm{act}} > 500$, condition (C1) is fulfilled. Therefore the model can indeed explain how T-cells are able to distinguish between self and non-self. Comparison with Fig. 3 of BRB [18] shows that the separation of the activation curves is indeed similar to that in the original model. In terms of the cartoon in Fig. 1, the threshold value g_{act} can be chosen so that T-cell 2 will be activated when it encounters APC 2 with three foreign peptides (the circles), while the other APCs without foreign peptides (the non-circles) will not activate any T-cell.



(a) $z_f = 0$, linear scale

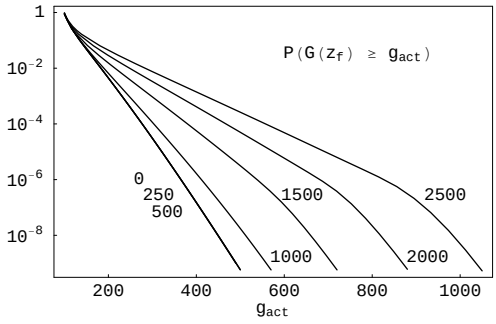


(b) $z_f = 0$, logarithmic scale

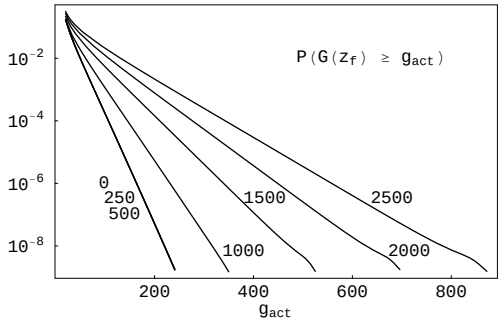


(c) $z_f = 2500$, logarithmic scale

Fig. 4 $\mathrm{P}(G(z_f) \geq g_{\mathrm{act}})$ as a function of g_{act}, for the self background ($z_f = 0$), and a very large number of foreign peptides ($z_f = 2500$). The thick black curve and the black points, respectively, form the simulated distribution of two million [(a),(b)] and twenty million [(c)] sampling points. The dashed curve is the normal approximation, and the grey curve is the large deviation approximation (23). The simulation in (c) was kindly provided by F. Lipsmeier.



(a) exponential distribution



(b) log-normal distribution

Fig. 5 Activation curves for values of z_f ranging from 0 to 2500, calculated according to approximation (23) for two different distributions for $\mathcal{T}_{ij}$. The horizontal axis is chosen to start at a value of g_{act} that yields a probability close to 1 in this approximation.

The intuitive reason behind the self versus non-self distinction is an elevated number of presented foreign peptides in comparison with the copy numbers of individual types of self-peptides. Indeed, this increases the variability of G (which is reminiscent of the fact that for $n \geq 2$ i.i.d. random variables with positive variance, nY_1 has a larger variance than $\sum_{i=1}^{n} Y_i$). So far the number of presented foreign peptides has to be fairly large: at least as large as the copy number of constitutive ones, which are, in turn, more abundant than the variable ones (one might actually reformulate the hypotheses pair (7) and (8) so as to

test whether the foreign antigen is more abundant than the constitutive peptides or not). However, this restriction vanishes when we take the training phase of the young T-cells into account, as will be done in the next subsection.

5.3 Activation curves with negative selection

In order to model the process called *negative selection* (see Jiang and Chess [10] for a recent review of the biological details), one postulates a second threshold g_{thy} with a similar role as g_{act}. If the stimulation rate of a young T-cell in its maturation phase in the thymus (where the APCs only present self-peptides) exceeds this threshold, then the T-cell is induced to die. For a caricature version of this process, let us assume that T-cell types present in the thymus exist in one copy each and encounter exactly one APC there.

The model then consists of two parts: first, the maturation phase is modelled to characterize the T-cell repertoire surviving negative selection; second, activation curves are calculated for this surviving repertoire. In the first step, we have to calculate the probability to survive negative selection conditional on the type of the T-cell:

$$\mathrm{P}(\text{survival of a T-cell of type } i) = \mathrm{P}\left(\sum_{j\in\mathcal{C}_i} Z_j^{(c)} w(\tau_{ij}) + \sum_{j\in\mathcal{V}_i} Z_j^{(v)} w(\tau_{ij}) < g_{\mathrm{thy}}\right) .$$

In this case the conceptual difference between $\mathcal{C}_i$ and $\mathcal{V}_i$ has an effect, which is essential. The constitutive types of peptides are the same on each APC, both in the thymus and in the rest of the body. Only these can be "learnt" as self by negative selection – the $\mathcal{V}_i$, being a fresh sample for every APC, are entirely unpredictable. Therefore we have

$$\mathrm{P}(\text{survival of a T-cell of type } i) = \mathrm{P}(\text{survival} \,|\, W_{ij} = w(\tau_{ij})\, \forall j \in \mathcal{C}_i) .$$

But in the case of fixed constitutive copy numbers z_c, the constitutive part of the stimulation rate reads $G^{(c)} = \sum_{j\in\mathcal{C}_i} z_c w(\tau_{ij})$, which is *constant* for fixed i. Therefore we have

$$\mathrm{P}(\text{survival} \,|\, W_{ij} = w(\tau_{ij})\, \forall j \in \mathcal{C}_i) = \mathrm{P}(\text{survival} \,|\, G^{(c)} = g_i) .$$

This simplifies the second step, the calculation of the activation curves conditional on survival: only a single integration step is required. Numerically, it turns out that (C1) is already fulfilled for $z_f \leq 500$ $(= \mathrm{E}(Z_1^{(c)}))$. Actually, the detection threshold for foreign antigens is reduced drastically (to about a third of the original value).

In our case (where the copy numbers vary from APC to APC), $G^{(c)} = \sum_{j\in\mathcal{C}_i} Z_j^{(c)} w(\tau_{ij})$, the constitutive part, varies from encounter to encounter. Indeed, whereas the $w(\tau_{ij})$ are fixed for each T-cell, the copy numbers are tied to the APCs. Therefore $\sum_{j\in\mathcal{C}_i} w(\tau_{ij})$ is not sufficient to determine $G^{(c)}$, and hence the survival probability; rather, the entire collection of the individual stimulation rates $w(\tau_{ij})$ for the constitutive types must be known to calculate the probability of the young T-cell to survive negative selection. The corresponding convolution required in the second step involves high-dimensional integrals, which appear to be computationally infeasible. In Van den Berg and Molina-Paris [20] this difficulty is tackled by simplifying the distribution of W to a Bernoulli variable. Here we resort to simulations.

To this end, we assume that each mature T-cell encounters the same number (in our simulation 1) of APCs in the rest of the body. For $g_{\mathrm{thy}} = 140$ (which for our choice of parameters corresponds to thymic deletion of about 5% of the young T-cells) the activation curves are shown in Fig. 6. As in the case of fixed copy numbers, we observe an incipient separation of the activation curves for $z_f = 0$ and $z_f = 500$. All in all, the above shows that the reduced detection threshold for foreign antigens occurs in the case of random copy numbers too. However, it seems that the separation of the activation curves is less pronounced here than in the case of constant copy numbers. This is plausible because the copy numbers of several constitutive peptides could be large (comparable to the copy number of the foreign peptide) and therefore the recognition does not work equally well. More pronounced separation of the

curves could be achieved by including mechanisms of so-called peripheral tolerance into the model. These mechanisms control the immune response of T-cells once they have left the thymus (mainly by the action of T-suppressor cells); see [10] for review and [20] for modelling approaches.

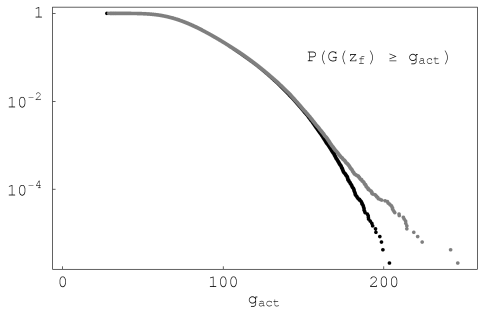


Fig. 6 Simulated activation curves with $g_{\text{thy}} = 140$ for $z_f = 0$ (black curve) and $z_f = 500$ (grey curve)

6 Discussion

We have analysed mathematically how T-cells can use large deviations to recognize foreign antigens. Before we discuss details of our results, let us pause here to discuss the notion of recognition that emerges from this analysis.

As explained in the introduction, the task the immune system faces is to recognize foreign antigens against a background of self-molecules. In much of the biological literature (and in line with intuitive understanding), this recognition is implied to be specific on a molecular basis. In sharp contrast, the mechanism proposed by BRB [18], and further analysed here, dispenses with such a high degree of strict specificity and, instead, is based on elevated copy numbers of the foreign antigen, relative to the self-background. This alleviates the Mason paradox about the repertoire size versus the universe of potentially relevant peptides. In this sense, specific recognition of antigen types is replaced by *probabilistic recognition* (this concept also appears elsewhere in biology, for example in the olfactory system; see, e.g., Lancet et al. [11]). We have examined this fact in detail for the situation without negative selection, where the principle becomes most transparent and can be largely dealt with via a large deviation analysis. Negative selection basically lowers the relevant background stimulation rate. Therefore it is easier for the foreign peptide to stand out against it, but the basic principle of recognition on the basis of frequencies remains the same.

The generalization we have examined in this article concerns precisely this fundamental issue of frequencies. In the original BRB-model [18], these frequencies were considered fixed (within the variable and constitutive class). In a subsequent paper, [20], a more sophisticated model of copy number variability was introduced (to reflect the fact that different cell types produce different proteins in different amounts, and some specialized cells may even produce large amounts of a variable peptide). We have replaced this here by a simpler approach that makes the analysis more amenable.

At the same time, allowing the Z_j to vary allows us to weaken assumption (A1), which is somewhat too restrictive in that it assumes that, for all $j \in \mathcal{P}_i$, all pMHCs are in the perfectly bound state. But if we simply reinterpret Z_j as the (fluctuating) number of *bound* antigens rather than the total number (i.e., corresponding to $\hat{c}_{ij}$ rather than to z_j), then we can circumvent (A1) and, at the same time, free ourselves from the details and limitations of the deterministic binding kinetics, the assumptions of which may sometimes be violated, for example, if copy numbers are low.

Although, in this vein, a detailed model of the binding kinetics may not be required, one last remark is in order concerning a potential *stochastic* model. Stochastic models of binding kinetics, e.g. as described in Ethier and Kurtz [6, Chapter 11], take care of the finite number of molecules, and of the resulting fluctuations of bound molecules over the short time scales of the binding kinetics. Over the longer time scales to be considered for our averaged stimulation rates, the corresponding time averages would be

relevant. In particular, these would in general not be integers; in this light, the factor q, which turns the multipliers of the sums in (6) into non-integer values, is rendered plausible.

Summarizing, our results show that the *probabilistic* recognition phenomenon is astonishingly robust against fluctuations of the copy numbers. Similarly, generalizations in various other directions that take into account many more biological details (see e.g. [20, 21, 23]), have shown that the phenomenon persists in a robust way. One may therefore hope that several details of the assumptions may actually be dispensed with. Future work will thus aim at identifying the joint mathematical content of the underlying family of models. In particular, this will include an analysis of what aspects of the distribution of the W's are key to the observed phenomenon. Luckily, the scope of the large deviation result Theorem 1 is wide enough to cope with very general situations. In particular, one is not bound to sums of independent random variables, but can also tackle dependencies (like, for example, those introduced by negative selection) within the present framework. Furthermore, the large deviation framework may also be used to construct efficient simulation methods for the tail events, as will be described in a forthcoming paper (F. Lipsmeier & E. Baake, in preparation).

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