Fluctuations in TCR and pMHC interactions regulate T cell activation

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ABSTRACT

Adaptive immune responses depend on interactions between T cell receptors (TCRs) and peptide major-histocompatibility complex (pMHC) ligands located on the surface of T cells and antigen presenting cells (APCs) respectively. As TCRs and pMHCs are often only present at low copy numbers their interactions are inherently stochastic, yet the role of stochastic fluctuations on T cell function is unclear. Here we introduce a minimal stochastic model of T cell activation that accounts for serial TCR-pMHC engagement, reversible TCR conformational change and TCR clustering. Analysis of this model indicates that it is not the strength of binding between the T cell and the APC cell *per se* that elicits an immune response, but rather the information imparted to the T cell from the encounter, as assessed by the entropy rate of the TCR-pMHC binding dynamics. This view provides an information-theoretic interpretation of T cell activation that explains a range of experimental observations. Based on this analysis we propose that effective T cell therapeutics may be enhanced by optimizing the inherent stochasticity of TCR-pMHC binding dynamics.

Introduction

Lymphocytes are responsible for immunity and a subset known as T cells are critical for adaptive immunity [1]. T cell receptors (TCRs) located on the T cell surface reversibly bind to peptide-major histocompatibility complex (pMHC) ligands located on the surface of antigen presenting cells (APCs) [2]. This interaction can activate the T cell leading to a variety of responses [3], including the production of soluble messengers called cytokines [4]. Furthermore, when activated a T cell is stimulated to proliferate, thereby generating progeny that can differentiate into effector cells [5]. These mature T cells are then able to clear antigen from the body by seeking out and destroying harmful pathogen-infected or tumor cells [6]. Although there have been a number of experimental and theoretical studies that have helped to uncover many of the mechanisms responsible for T cell activation [7, 8, 2, 6, 9], it is still unclear which properties of the TCR-pMHC interactions determine the T cell response.

In the late 1990s and early 2000s a series of landmark studies suggested that three mechanisms are key. First, Valitutti *et al.* [10, 11] proposed that just a few pMHC ligands can serially bind many TCRs and that this serial engagement leads to a conformational change in each TCR. Second, a number of authors subsequently showed that TCR conformational change is both directly induced by pMHC ligand binding and reversible [12, 13, 14, 15, 9]. Third, Monks *et al.* found that TCRs can also cluster into what they called the central supra-molecular activation cluster (c-SMAC) following pMHC binding [16]. It has since been shown that TCRs can form 'microclusters' upon pMHC binding prior to coalescing at the c-SMAC [17, 18, 19]. These three mechanisms: serial TCR-pMHC engagement; reversible conformational TCR change; and TCR clustering are

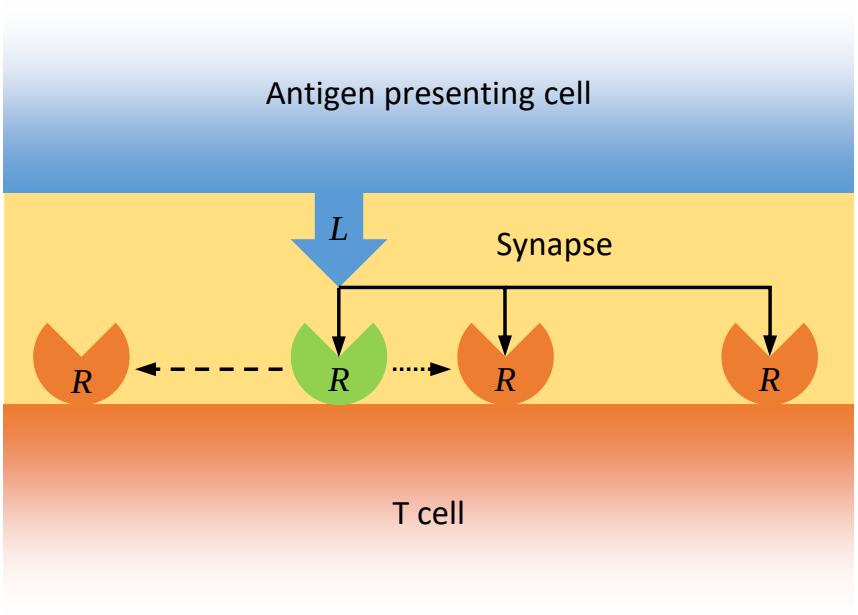

Figure 1. Schematic of mechanisms involved in TCR-pMHC binding. Three key mechanisms are highlighted: (1) Solid arrows represent a pMHC ligand serially engaging with multiple TCRs within the synapse. (2) The green TCR represents an induced conformational change upon pMHC ligand binding. The dashed arrow represents the TCR reverting back to its original state following unbinding. 3) The dotted arrow represents TCR clustering, whereby TCRs that have undergone pMHC binding coalesce to form microclusters and subsequently the c-SMAC within the synapse.

shown schematically in **Fig. 1**.

Notably, both conformational TCR change and TCR clustering have been shown to be necessary for T cell activation [20] and may also improve antigen discrimination [21, 22]. Moreover, it has been suggested that a combination of all three mechanisms may allow the T cell to efficiently scan the APC surface with high specificity and sensitivity for rare pMHC ligands presented at low copy numbers [23, 24, 7, 1, 8, 3].

Indeed, there is increasing evidence that T cell activation can be induced by as few as ∼1-10 pMHC ligands [25, 26, 27] and that microclusters may contain as few as ∼10-100 TCRs [17, 18, 19, 28, 23, 27]. At such low copy numbers the TCR-pMHC binding dynamics are inherently stochastic, yet the effect of this stochasticity on T cell activation is unclear. Stochastic fluctuations have been shown to be functionally important in numerous other biological contexts [29, 30, 31] and, therefore, it is conceivable that the T cell has evolved to utilize these fluctuations to enhance its own function.

Here, we develop a minimal stochastic model of the TCR-pMHC binding dynamics that includes serial TCR-pMHC engagement, reversible conformational TCR change and TCR clustering. We show that these three mechanisms collectively allow the T cell to convert stochastic fluctuations in the TCR-pMHC binding dynamics into a well-defined signal. Based on this analysis we propose that the T cell response to an APC is not determined by the strength of TCR-pMHC binding *per se*, but rather by the information conveyed to the T cell by the encounter, as assessed by the entropy rate of the TCR-pMHC binding dynamics. We test this hypothesis against a range of experimental studies, including a comprehensive dose-response data-set [32], before discussing the implications for T cell based therapeutics.

Results

Fluctuations in TCR-pMHC binding dynamics generate information

To start, we will introduce some information-theoretic notions in the context of a simple model of TCR-pMHC binding, before discussing how they apply to a more realistic model of T cell activation.

Consider the process of TCR-pMHC reversible heterodimerization, given by the following reactions:

$$L + R \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} B\,, \tag{1}$$

where L denotes the pMHC ligand, R denotes the TCR, B denotes the TCR-pMHC complex, k_{off} is the rate of unbinding, k_{on}/ν is the rate of binding [33] and ν is the 2-dimensional (2D) contact area in which the biochemical reactions take place.

At low copy numbers these reactions will be inherently stochastic and the copy number of the TCR-pMHC complex will accordingly fluctuate randomly over time. To quantify the extent of this stochasticity we will use two measures. First, the Shannon entropy, $H(B)$ (in bits) given by:

$$H(B) = -\sum_{i=0}^{B_{\text{max}}} p(i)\log_2 p(i), \tag{2}$$

where B_{max} is the maximum number of complexes (given by Eq. 11 in the **Methods**) and $p(i)$ is the stationary probability that i copies of the TCR-pMHC complex are present (given by Eq. 13 in the **Methods**). In what follows, we will assume that the T cell responds on a slower timescale than the TCR-pMHC binding dynamics, and consider properties of stationary probability distributions only. In general, the Shannon entropy is a simple measure of information or 'disorder' [34]. In the context of the T cell-APC synapse, it is the average amount of information imparted to the T cell per TCR-pMHC binding/unbinding event. As such, although it is a useful measure of information, the Shannon entropy does not take account of the speed of the underlying reactions which will vary with the kinetic rate parameters. Therefore, the Shannon entropy cannot distinguish between fast and slow dynamics.

To clarify this distinction we will use an alternative measure: the entropy rate, $H'(B)$ (in bits per second) which is calculated as the mean reaction rate (i.e. the average number of binding/unbinding events per second) multiplied by the Shannon entropy. For the reversible heterodimerization reactions given in Eq. 1, the entropy rate is:

$$H'(B) = 2k_{\text{off}}\langle B\rangle H(B), \tag{3}$$

where $\langle B\rangle$ is the mean number of TCR-pMHC complexes at equilibrium (for details see the **Supplementary Information**). In the context of the T cell-APC synapse, the entropy rate is the average amount of information imparted to the T cell by the TCR-pMHC binding dynamics per second. Therefore, unlike the Shannon entropy, the entropy rate can distinguish between fast and slow dynamics.

While it has a useful information-theoretic interpretation, the entropy rate is complex to calculate in practice. However, we can similarly define the 'variance rate', $\text{Var}'(B)$ as:

$$\text{Var}'(B) = 2k_{\text{off}}\langle B\rangle \,\text{Var}(B), \tag{4}$$

where $\text{Var}(B)$ is the variance of the TCR-pMHC complex stationary distribution. Although the variance rate does not have an information theoretic interpretation, it exhibits similar features to the entropy rate for the simple dynamics described here

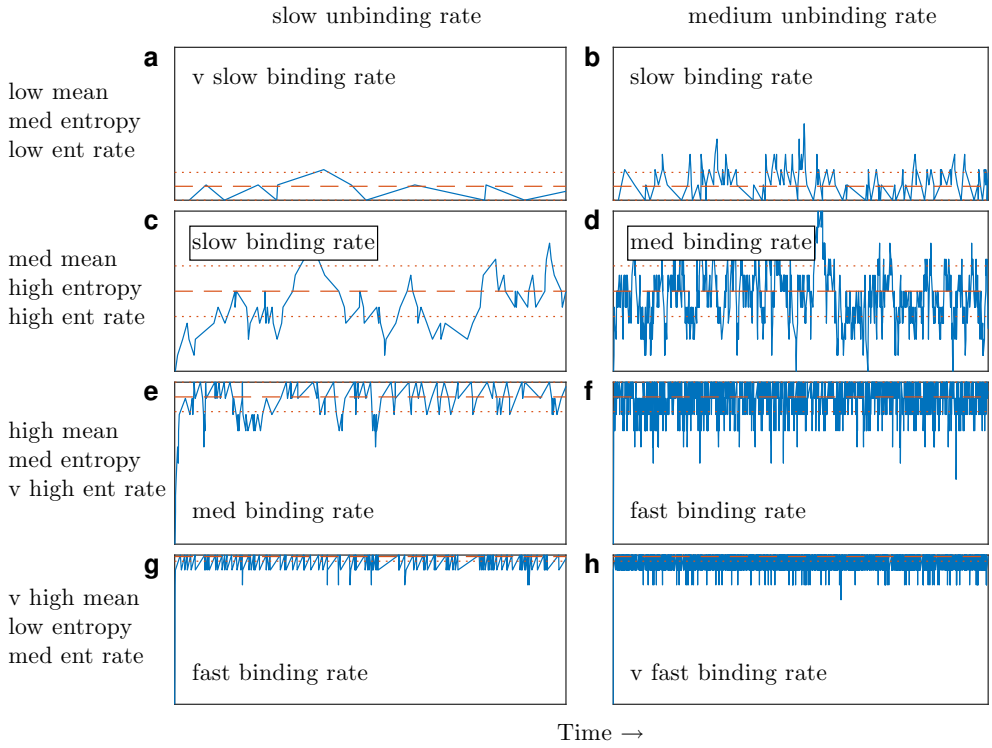

Figure 2. Fluctuations in TCR-pMHC dynamics generate information Representative stochastic simulations of the TCR-pMHC complex copy number, B for the first 10 seconds of the reversible heterodimerization reactions given in Eq. 1. Dashed red lines show the mean and dotted red lines show the mean plus/minus one standard deviation. In all panels the maximum number of TCR-pMHC complexes, B_{max} given by Eq. 11, is set to 10. All other parameters are varied over orders of magnitude within a plausible physiological range as described in the **Methods**.

and is more analytically tractable. We will make use of this connection in the next section, where we analyze a more realistic model of TCR-pMHC binding dynamics.

To illustrate these concepts, **Fig. 2** shows some representative stochastic simulations of TCR-pMHC reversible heterodimerization using Gillespie's direct method [35, 36]. Three features of these simulations are notable.

First, while the mean number of TCR-pMHC complexes increases monotonically with the binding rate (k_{on}/ν), both the Shannon entropy and the entropy rate initially increase as the binding rate increases, but then decrease as the binding rate increases further still (to see this compare the panels in each column of **Fig. 2**). This biphasic pattern occurs because fluctuations are minimal when binding is very weak or very strong (i.e., when complexes do not easily associate or dissociate respectively) yet become strong at intermediate affinities that allow both binding and unbinding events to easily occur.

Second, while the mean number of TCR-pMHC complexes and Shannon entropy are dependent on three model parameters: the total number of pMHC ligands and TCRs at the contact area (which we denote L_{max} and R_{max} respectively) and the 2D dissociation constant, K_{d} given by:

$$K_{\text{d}} = \frac{\nu k_{\text{off}}}{k_{\text{on}}}; \tag{5}$$

the entropy rate is explicitly dependent on both the binding rate and the unbinding rate (rather than simply the ratio of the two, K_{d}). Thus, dynamics associated with different kinetic rate parameters may have the same mean number of TCR-pMHC complexes and Shannon entropy, but very different entropy rates (to see this compare the panels in each row of **Fig. 2**. In each

case, the entropy rate in the right column is an order of magnitude higher than in the left column).

Third, for a fixed unbinding rate, the maximum entropy rate (and therefore the maximum rate at which information can be imparted to the T cell) is achieved via a trade-off between the average number of bound complexes and average magnitude of the stochastic fluctuations. So, the TCR-pMHC binding dynamics illustrated in **Fig. 2f** have the largest entropy rate of all the panels because they combine both a relatively high mean with a relatively high Shannon entropy.

Collectively, this reasoning suggests that fluctuations in TCR-pMHC binding dynamics can generate information and thereby may have an important, but as yet unexplored, part to play in regulating T cell activation.

TCR-pMHC fluctuations determine T cell activation

To investigate this possibility further we sought to construct a minimal model of the TCR-pMHC binding dynamics that includes the effects of serial engagement and reversible conformational change. Our minimal model consists of the following set of reactions:

$$L + R_{\mathrm{I}} \xrightarrow{k_{\mathrm{on}}} B\,, \tag{6}$$

$$B \xrightarrow{k_{\mathrm{off}}} L + R_{\mathrm{A}}\,, \tag{7}$$

$$L + R_{\mathrm{A}} \xrightarrow{k_{\mathrm{on}}} B\,, \tag{8}$$

$$R_{\mathrm{A}} \xrightarrow{k_{\mathrm{r}}} R_{\mathrm{I}}\,, \tag{9}$$

where R_{I} and R_{A} denote 'inactive' TCRs and 'active' TCRs respectively (note that an active TCR is one that has undergone a conformational change due to pMHC ligand binding; see **Fig. 1**). Additionally, we model the generation of an activating T cell signal, S, due to TCR clustering with the following reaction:

$$R_{\mathrm{A}} + B \xrightarrow{k_{\mathrm{s}}} R_{\mathrm{A}} + B + S\,. \tag{10}$$

We emphasize that equations 6–10 are not meant to be a detailed model of every aspect of TCR-pMHC binding and T cell activation. Rather, they encapsulate key mechanisms in a parsimonious way that allows for a transparent exploration of their consequences. Particularly, equation 10 captures salient features of TCR aggregation without recourse to complex stochastic reaction-diffusion processes which, although more mechanistically detailed, may be less tractable and harder to interpret [37]. A detailed explanation of how each reaction relates to each of the three driving mechanisms detailed in **Fig. 1** is provided in the **Methods**.

This modeling framework is useful because it accounts for additional mechanisms of importance, yet central aspects of the reversible heterodimerization reactions given in Eq. 1 are conserved (for details see the **Supplementary Information**). In particular, the dynamics of the TCR-pMHC complex number, B, pMHC ligand number, L, and the sum of the inactive and active TCR numbers, $R = R_{\mathrm{I}} + R_{\mathrm{A}}$, are equivalent to those of the straightforward reversible heterodimerization reactions. Thus, calculations of the mean number of TCR-pMHC complexes, the Shannon entropy, and the variance/entropy rates described in the previous section also apply to this model.

Moreover, the effects of these quantities on signal generation may now be explored. In the **Supplementary Information** we show that the mean number of active TCRs in the contact area, $\langle R_{\mathrm{A}} \rangle$, is equal to the variance of the TCR-pMHC complex number, $\mathrm{Var}(B)$, in wide regions of parameter space. This is notable because in this framework a T cell signal is (stochastically) generated if an active TCR is in close proximity to a TCR-pMHC complex (see Eq. 10). Consequently, this implies that the mean signaling rate, $\langle \dot{S} \rangle$ (i.e. the average rate at which a signal is generated) is approximately equal to the variance rate, $\mathrm{Var}'(B)$, for a wide range of parameter values. Thus, fluctuations in TCR-pMHC binding dynamics drive T cell activation. Details of the

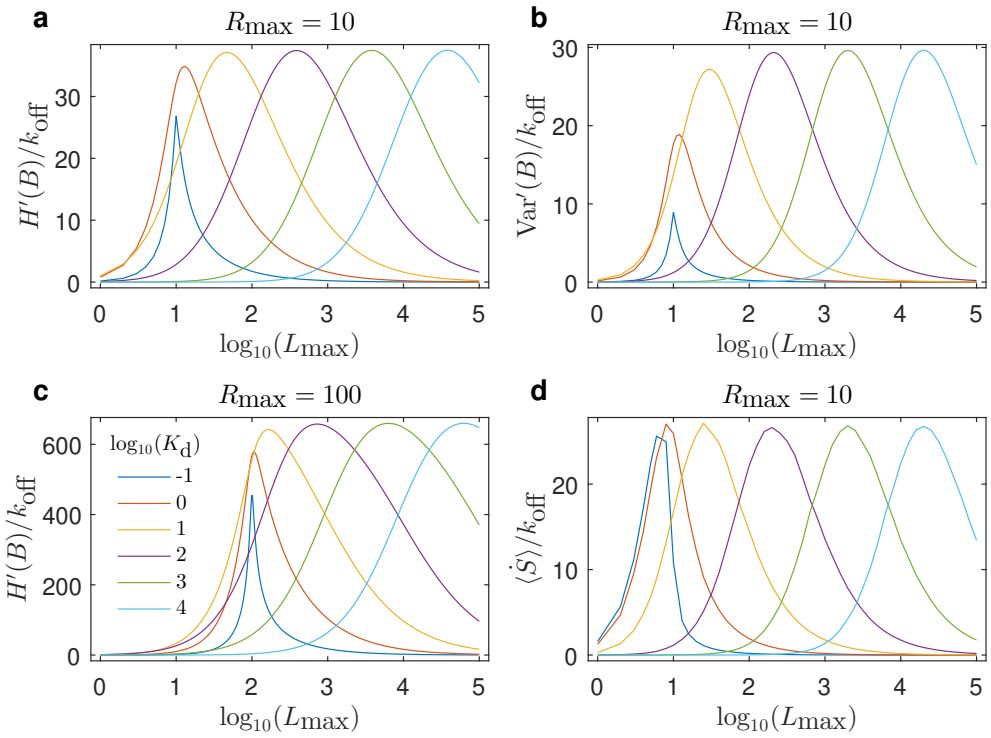

Figure 3. T cell signaling rate is determined by TCR-pMHC fluctuations Entropy rate (**a,c**) and variance rate (**b**) of the stationary TCR-pMHC complex copy number dynamics as a function of the number of pMHC ligands. Mean signaling rate (**d**) based on the minimal model of T cell activation as calculated via stochastic simulations of the reactions given in Eqs. 6–10. Comparison of panels **a** and **c** shows that variation in the total number of TCRs does not affect the qualitative nature of the curves. In all panels time has been rescaled by k_{off} to provide curves that vary by affinity, which allows for a direct comparison with experimental dose-response data. Entropy rate, variance rate and mean signaling rate are therefore expressed per mean lifetime of a TCR-pMHC complex, rather than per second. Parameter values in all simulations were determined from the literature. Further details of parameters are provided in the **Methods**. All presented values are dimensionless.

mathematical derivations of these results are provided in the **Supplementary Information**, and illustrated numerically in **Fig. 3**.

Significantly, all three TCR-proximal mechanisms (serial engagement, reversible conformational change and receptor clustering) are required to generate a signal at a rate determined by the variance rate of the TCR-pMHC binding dynamics (see **Supplementary Information** for details). Thus, in the absence of any one of these mechanisms the T cell is not able to convert stochastic fluctuations in TCR-pMHC binding dynamics on the cell surface into a defined cellular response; yet, when all three are present, it can. This reasoning suggests that these mechanisms work collectively to allow the T cell to process noisy environmental information appropriately.

In addition to offering an explanation of why these mechanisms are central to T cell activation, this perspective has important implications for optimization of the T cell response. In this model, increasing the TCR-pMHC binding rate initially increases the variance of the TCR-pMHC complex number and thereby the mean number of active TCRs and mean signaling rate (starting from a low binding rate). However, as the binding rate increases further still, the variance of the TCR-pMHC complex number will fall and, while the mean number of TCR-pMHC complexes will continue to increase, the mean number of active TCRs will start to decrease. This, in turn, leads to a decrease in the mean signaling rate. Thus, because T cell activation is not determined solely by the binding strength (i.e. affinity) between the TCR and pMHC molecules, but also by dynamic fluctuations, maximal

T cell activation is predicted to occur at an intermediate affinity, for a fixed number of pMHC ligands (i.e. dose). For similar reasons, maximal T cell activation is also predicted to occur at an intermediate dose, for a fixed affinity. **Fig. 3** shows numerical simulations of binding and activation dynamics that illustrate these points (also discussed further in the following section).

Interestingly, a number of experimental studies have reported that the T cell response is indeed maximized at an intermediate affinity [38, 39, 40, 41, 42, 43]. However, the reason for this optimization has not been clear. Our model suggests that it occurs because intermediate affinity and intermediate dose scenarios both give rise to highly stochastic, information-rich, dynamics which the T cell is able to process, via simple molecular mechanisms, into a defined cellular response.

Collectively, these results indicate that stochastic fluctuations, as quantified by the variance rate of the TCR-pMHC binding dynamics, may regulate T cell activation. While we could not obtain a corresponding analytical result for the entropy rate, the variance rate and entropy rate are very closely related and numerical simulations of TCR-pMHC binding dynamics indicate a similar dependency (*cf* **Fig. 3a** and **Fig. 3b**). This suggests that the variance rate is an analytically convenient proxy for the more biologically-meaningful entropy rate as a measure of TCR-pMHC fluctuation strength.

Experimental evidence

To investigate the validity of this view we sought to determine further experimental support. To do so, we considered the data of Lever *et al.* [32], which is arguably the most detailed dose-response study of T cell activation to-date and so provides a comprehensive data set to benchmark our predictions. In their experiments, Lever *et al.* [32] assessed T cell response, determined by cytokine production, to varying concentrations of pMHC dose and changes in 3-dimensional affinity, as measured by surface plasmon resonance [5]. Both inputs were varied over six orders of magnitude, while TCR concentration was assumed to remain constant. They summarized the results of their extensive experiments in the following four statements concerning T cell activation:

1. Dose-response curves are bell-shaped for high- but not low-affinity pMHC ligands.
2. The peak amplitude of bell-shaped dose-response curves is independent of affinity.
3. A single intermediate affinity pMHC ligand produces largest response at low pMHC doses.
4. Different intermediate affinity pMHC ligands produce the largest response at high pMHC doses.

All four of these statements are consistent with our interpretation, in which the variance/entropy rate of the TCR-pMHC binding dynamics can be considered as the 'response'. **Fig. 3** shows the variance rate, entropy rate and mean signaling rate for a range of pMHC doses and affinities for comparison with the results of Lever *et al*. Since it has a defined information-theoretic meaning, below will focus on the entropy rate as a proxy for T cell response. Similar results may be also be seen for the variance and signaling rates (**Fig. 3b, d**)

From this figure it is apparent that the entropy rate of TCR-pMHC binding dynamics is bell-shaped for intermediate to high affinity pMHC ligands (i.e. the red, yellow, purple and green curves in **Figs. 3a, c**). This bell-shape is truncated for the lowest affinity pMHC ligand (in light blue) due to the upper end of the pMHC dose range, giving the appearance of a sigmoidal curve (see **Fig. 3c** in particular); a similar phenomenon was observed by Lever *et al*. Moreover, the peak amplitude of the entropy rate is broadly independent of affinity, particularly for low to intermediate affinity pMHC ligands (i.e. the light blue, green, purple and yellow curves in **Figs. 3a, c**). Only the very highest pMHC ligand affinity (dark blue) shows a peak amplitude that is clearly different to the others. Similarly, a single intermediate affinity pMHC ligand produces the largest entropy rate at low pMHC doses, while different intermediate affinity pMHC ligands produce the largest entropy rate for large pMHC doses (i.e. compare the red curve with the yellow, purple and green curves in **Fig. 3a**).

Collectively, these results indicate that simple information theoretic reasoning can help interpret complex dose-response data, and suggest that T cell response is determined by TCR-pMHC fluctuations.

Discussion

A general communication system consists of at least three interconnected parts: an information source, a channel and a destination [44]. In the context of T cell activation, TCR-pMHC binding dynamics can be thought of as the information source; intracellular signaling pathways as the channel; and the cell nucleus as the destination. From this perspective, stochasticity in TCR-pMHC binding dynamics generates a 'message' which, depending on the kinetic rate parameters, may contain more or less information. Moreover, the average information content per second of this message, as assessed by the entropy rate, represents the average rate at which peptide-specific information is conveyed to the nucleus via signaling pathways. Based on this reasoning, we hypothesize that T cell activation is regulated by the entropy rate of the TCR-pMHC binding dynamics. More generally this reasoning suggests that tools from information theory may help to shed light on the complex information processing mechanisms involved in T cell activation. Important work in this area, for example to better understand antigen discrimination, has already begun [45].

This simple idea explains a wide variety of experimental data, without recourse to complex or putative mechanisms. For example, to explain the dynamics they observed (summarized in the four statements above), Lever *et al.* [32] argued that both kinetic proof-reading and a putative incoherent feed-forward loop which acts downstream of the TCR are required. They identified such a minimal model based on a deterministic framework that accounts for average expression levels but not fluctuations. In contrast, by taking a stochastic view – and reorienting our perspective on the mechanistic basis of T cell response to include a role for the information contained in stochastic fluctuations – our model is able to explain these data, using well-established mechanisms. This does not mean, of course, that more complex regulatory mechanisms are not present in T cell activation dynamics (they certainly are, and mechanisms such as kinetic proof-reading and processing by regulatory motifs considered by Lever *et al* are undoubtedly important), but rather that they may not be required to explain much experimental evidence.

The implications of these considerations are perhaps most important for designing the next generation of immunotherapies. For example, identifying the optimal affinity and dose is central to the design of both vaccines [41, 42] and chimeric antigen receptor (CAR) T cell therapies [46, 47, 48, 49]. Our information-theoretic approach provides a framework to guide the optimization of the T cell response via modification of the dose or affinity of the TCR-pMHC binding dynamics. This issue is considered further in the **Supplementary Information**, where we provide a numerical procedure to calculate the optimal affinity under conditions in which both the total number of TCRs and pMHC ligands are fixed. More speculatively, if it were possible to manipulate both the binding and unbinding rates then our analysis suggests that the T cell response will increase with faster kinetics, providing that the optimal affinity is maintained.

Our results also provide a note of caution. Shannon's seminal information theorems [44] show that it is unproductive for the entropy rate of a message to exceed the communication system's channel capacity, because the channel capacity sets an upper limit to the rate of information transmission. This suggests that there is a limit to the rate at which the T cell can process information, which is set by the intracellular signaling pathways that transmit signals from the cell surface to the nucleus. Thus, there may be a limit to our ability to engineer T cell therapeutics based on manipulation of the TCR-pMHC kinetic rate parameters, unless the capacity of the signaling pathway(s) that transmit these messages can also somehow be increased. To quote Lombardi *et al.* [50] '*The goal in the field of communication engineering is to optimize the transference of information through channels conveniently designed*'. We speculate that the same may be true for T cell engineering.

Although we have focused on the T cell response, the simplicity of our perspective means that an information-theoretic perspective of receptor-ligand binding could have application to a wide range of other therapeutics. For instance, experimental evidence for binding-induced conformational change that subsequently induces clustering not only exists for the T cell receptor [51] but has also been found for the B cell receptor [52, 53].

Methods

Fluctuations in the TCR-pMHC binding dynamics

In the context of the reactions of Eq. 1, let $B_{\max}$ and $U_{\max}$ denote the smaller and larger respectively of the total number of pMHC ligands, $L_{\max}$, and total number of TCRs, $R_{\max}$, given by:

$$B_{\max} = \min(L_{\max}, R_{\max}), \tag{11}$$

$$U_{\max} = \max(L_{\max}, R_{\max}). \tag{12}$$

Note that $B_{\max}$ is also the maximum number of TCR-pMHC complexes. The stationary distribution of the TCR-pMHC complex number, $p(B)$ is given by:

$$p(B; B_{\max}, U_{\max}, K_{\mathrm{d}}) = \frac{a(B; B_{\max}, U_{\max}, K_{\mathrm{d}})}{Z(B_{\max}, U_{\max}, K_{\mathrm{d}})}, \tag{13}$$

where

$$a(B; B_{\max}, U_{\max}, K_{\mathrm{d}}) = \binom{B_{\max}}{B} \binom{U_{\max}}{B} {K_{\mathrm{d}}}^{-B} B!, \tag{14}$$

$$Z(B_{\max}, U_{\max}, K_{\mathrm{d}}) = \sum_{i=0}^{B_{\max}} a(i; B_{\max}, U_{\max}, K_{\mathrm{d}}), \tag{15}$$

and where K_{d} is given by Eq. 5. The probability of there being at least one TCR-pMHC complex in the contact area, P_{a} is given by:

$$P_{\mathrm{a}} = 1 - \frac{1}{Z(B_{\max}, U_{\max}, K_{\mathrm{d}})}. \tag{16}$$

Eq. 16 is commonly referred to as the 'probability of adhesion' between two cells [23]. Full details of these and further calculations are provided in the **Supplementary Information**.

Model parametrization

To produce the simulations provided in **Fig. 2** and **Fig. 3** we used parameters from the literature. Specifically, Huang *et al.* [23] fitted an approximation to the probability of adhesion, P_{a} given by Eq. 16, to data derived from $CD8^+$ OT1 T cells and APCs pulsed with a range of peptides. Using their descriptions but our notation, their two fitted parameters were the 'effective 2D affinity', ν^2/K_{d}, and the '2D off rate', k_{off}. Their three measured parameters were the 'adhesion frequency', $P_{\mathrm{a}}(t)$, the 'pMHC density', $L_{\max}/\nu$, the 'TCR density', $R_{\max}/\nu$, and the '2D contact area', ν. The 'effective 2D on rate', νk_{on} was then calculated as the product of the effective 2D affinity and the 2D off rate (*cf* Eq. 5). The 2D contact area was described as 'a few percent' of 3 $\mu\mathrm{m}^2$ for micropipette and 1 $\mu\mathrm{m}^2$ for biomembrane force probe. To utilize these parameter assessments, we used an order of magnitude estimate of $\nu \sim 10^{-1}\mu\mathrm{m}^2$ for converting to our dimensionless parameters of $L_{\max}$, $R_{\max}$ and K_{d} as well as k_{on}/ν. Based on experiments performed at 37 °C the effective 2D affinity had an estimated range of $\nu^2/K_{\mathrm{d}} \in [10^{-3}, 10^{-6}]\mu\mathrm{m}^4$ and the 2D off rate had an estimated range of $k_{\mathrm{off}} \in [10^0, 10^1]\mathrm{s}^{-1}$. This gives an order of magnitude range of $K_{\mathrm{d}} \in [10^1, 10^4]$ and $k_{\mathrm{on}}/\nu \in [10^{-4}, 10^0]\mathrm{s}^{-1}$. Given the potential for engineered therapeutic TCRs that bind with high affinity [32], we extended this range of K_{d} and k_{on}/ν for our calculations in order to capture plausible physical values. The range of the pMHC density was measured as $L_{\max}/\nu \in [10^1, 10^3]\mu\mathrm{m}^{-2}$ which gives an order of magnitude range of $L_{\max} \in [10^0, 10^2]$. Lever *et al.* [32] were able to vary the dose by a million-fold in their dose-response experiments so we extend the range of $L_{\max}$ for our calculations,

again to capture plausible physical values. Huang *et al.* also measured the TCR density as $R_{\max}/\nu \sim 10^2 \mu\text{m}^{-2}$ giving our parameter estimate of $R_{\max} \sim 10^1$. Other studies have found that the number of TCRs in individual microclusters and at the c-SMAC is $\sim$ 10-100 [17, 18, 19, 23, 27]. Therefore, we also considered $R_{\max} \sim 10^2$ in **Fig. 3c** as a sensitivity analysis.

Minimal model of signal generation

Equations 6, 7, 8 and 9 model a combination of the serial TCR-pMHC engagement and TCR reversible conformational change mechanisms. Specifically, equations 6 and 7 model an inactive TCR (i.e. a TCR in its resting state), R_{I}, undergoing a conformational change upon pMHC ligand binding [12, 13, 14] and entering an active state, R_{A}, upon unbinding. The active state of the TCR represents a form of memory following pMHC ligand binding [54, 8, 55]. For simplicity we assume that an active TCR can bind with a pMHC ligand at the same rate as an inactive TCR as shown by Eq. 8. Equation 9 models an active TCR reverting to an inactive TCR at a rate k_{r} [12, 13, 14, 9]. Equation 10 models the TCR clustering mechanism (either at the c-SMAC [16] or in a microcluster [17, 18, 19]) whereby a signal is generated at a rate k_{s}/ν providing that an active TCR is in sufficient proximity to a TCR-pMHC complex.

The mean signaling rate shown in **Fig. 3d** was calculated via repeated stochastic simulations as follows. Initial conditions were: $R_{\text{I}}(0) = R_{\max} = 10$ and $R_{\text{A}}(0) = B(0) = S(0) = 0$. The rate constants were taken to be $k_{\text{off}} = k_{\text{r}} = k_{\text{s}}/(2\nu) = 1/\text{s}$. In addition, k_{on}/ν was varied between 10^{-4}/s and 10/s to give the values of K_{d} shown in the legend as calculated via Eq. 5. Each stochastic simulation was run until either $S_{\text{sim}} > 10^4$, or $t_{\text{sim}} > 10^4$s and then the signaling rate was calculated as $\dot{S} = S_{\text{sim}}/t_{\text{sim}}$. The mean signaling rate was then calculated as the mean of $\dot{S}$ over 10 simulations for each set of parameter values.

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