Feedback control of T-cell receptor activation

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The specificity and sensitivity of T-cell recognition is vital to the immune response. Ligand engagement with the T-cell receptor (TCR) results in the activation of a complex sequence of signalling events, both on the cell membrane and intracellularly. Feedback is an integral part of these signalling pathways, yet is often ignored in standard accounts of T-cell signalling. Here we show, using a mathematical model, that these feedback loops can explain the ability of the TCR to discriminate between ligands with high specificity and sensitivity, as well as provide a mechanism for sustained signalling. The model also explains the recent counter-intuitive observation that endogenous 'null' ligands can significantly enhance T-cell signalling. Finally, the model may provide an archetype for receptor switching based on kinase-phosphatase switches, and thus be of interest to the wider signalling community.

Keywords: T-cell receptors; cellular activation; signal transduction

1. INTRODUCTION

CD4⁺ T cells can be activated by the presence of 100–200 foreign peptide–major histocompatibility complexes (MHCs) on the surface of an antigen presenting cell (APC), out of 10⁵–10⁶ total peptide–MHC complexes (Germain & Štefanová 1999). CD8⁺ T cells are even more sensitive, and may be capable of responding to a single foreign peptide–MHC ligand (Sykulev *et al.* 1996). This suggests that T-cell receptors (TCRs) must have extremely high specificity for ligand discrimination, because otherwise the signals from self ligands will swamp those from foreign ligands. This specificity is clearly seen in the ability of T cells to discriminate between altered peptide ligands (APLs) differing at only a single amino acid residue (Sloan-Lancaster *et al.* 1994). For example, Kersh *et al.* (1998) reported that a difference in binding time of *ca.* 30% (equivalent to a 3 s decrease in $t_{1/2}$) can result in a 1000-fold difference in the biological potency of the ligand, whereas a 75% difference (*ca.* 7–8 s) results in a 16 000-fold difference. It is also clear that the ligand dissociation time alone does not determine potency, because the same study reported an APL with a 50% difference in binding time (*ca.* 5 s) had only a 50-fold difference in potency. Even so, a 50-fold variation in potency is a dramatic difference for ligands with only a 5 s difference in $t_{1/2}$.

A widely accepted model to explain this specificity and sensitivity is the kinetic proofreading model (McKeithan 1995). This assumes that when a TCR engages its ligand, the complex undergoes an obligatory sequence of modifications, and signals only when it has completed this sequence. This introduces a delay between a ligand first binding to the TCR and its subsequent activation. Ligands with high off-rates are likely to dissociate (hence

giving no signal) before this sequence is complete. Therefore only those ligands that bind for sufficiently long to the TCR can activate it.

It is clear that TCR activation is an immensely complex process with multiple molecular signalling events. However, only those events that occur before ligand dissociation can contribute to kinetic proofreading. Within the time-scale for TCR activation (several seconds), the only well-documented signalling events are the recruitment and activation of the Src-family kinases Lck and Fyn. The adapter Nck has also recently been reported to be recruited early (Gil *et al.* 2002), but the significance of this to TCR activation is not yet clear. In addition, unless immunoreceptor tyrosine-based activation motif (ITAM) phosphorylation occurs in a *serial* order, it cannot contribute significantly to specificity enhancement by kinetic proofreading (Chan *et al.* 2003). That ITAM phosphorylation may not contribute much to ligand discrimination is also suggested by experiments where TCRs without functional ζ -chains can still discriminate between antagonist and agonist ligands (Ardouin *et al.* 1999). This has been discussed in more detail in a recent publication (Chan *et al.* 2003). In this paper, we propose an alternative mechanism for kinetic proofreading, taking into account the current understanding of the signalling mechanisms involved in T-cell activation.

The interaction between the TCR and its agonist peptide–MHC complex is generally short-lived, of the order of several seconds. Partial agonist and antagonist ligands have even more rapid dissociation times (Davis *et al.* 1998). It is believed that the most proximal signalling event after TCR triggering by ligand is the activation of Src family kinases, specifically Lck and Fyn. These phosphorylate the ITAMs on CD3, resulting in the recruitment and activation of ZAP-70. ZAP-70 then phosphorylates several downstream adaptor proteins, eventually resulting in the activation of the phosphatidylinositol and Ras pathways.

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Because Lck and Fyn are activated within seconds after TCR cross-linking, whereas peak Syk and ZAP-70 activity takes at least 5 min (Burkhardt *et al.* 1994), it is likely that ligand discrimination involves Lck and Fyn activation. This is supported by the finding that antagonists and partial agonists fail to phosphorylate and activate ZAP-70 (Sloan-Lancaster *et al.* 1994; Madrenas *et al.* 1995). We concentrate on Lck in our models because Lck knockout mice show severe peripheral T-cell signalling deficiencies, while peripheral T-cell signalling in Fyn knockouts is relatively intact (Molina *et al.* 1992; Stein *et al.* 1992).

The regulation of Lck is complex. Like all Src kinases, it has SH2, SH3 and catalytic domains between the N- and C-terminal regions. The activity of Lck is regulated by two critical tyrosine residues (Sicheri & Kuriyan 1997). Phosphorylation of the negative regulatory C-terminal tyrosine (Y505) residue by Csk results in a low-affinity intra-molecular association with the SH2 domain and an inactive conformation. Dephosphorylation of this residue by CD45 results in a 'primed' Lck capable of being activated by *trans* auto-phosphorylation of the positive regulatory tyrosine (Y394) in the catalytic domain. However, in resting T cells, most of the Lck is already dephosphorylated at the inhibitory Y505 (Ostergaard *et al.* 1989; McFarland *et al.* 1993). This suggests that the primary events in T-cell activation and ligand discrimination involve Lck recruitment and auto-phosphorylation at Y394.

Lck in the membrane of resting T cells associate with a coreceptor. However, there is a significant pool of non-coreceptor-associated Lck (Bonnard *et al.* 1997). Because TCRs can be activated in the absence of coreceptors (Abraham *et al.* 1991), the association between CD4 or CD8 and Lck is not essential for its kinase activity. In fact, it appears that CD4 and CD8 are recruited to the TCR by an interaction between the SH2 domain of Lck and ζ -associated phosphorylated ZAP-70 (Thome *et al.* 1995, 1996), i.e. *after* an initial coreceptor independent activation of the TCR/CD3 complex. CD4 may function mainly to deliver Lck to the T cell : APC contact area, and dissociate from Lck after activation (Holdorf *et al.* 2002), although this is controversial (Ehrlich *et al.* 2002).

Because Lck activity depends on the phosphorylation states of its regulatory tyrosine residues, it is probable that an activated Lck molecule remains in this same state until a later deactivation event occurs. Mutational analysis suggests that the phosphorylation of Y394 is dominant to that of Y505 in determining the kinase activity of Lck (D'Oro *et al.* 1996), and therefore the deactivation event probably involves dephosphorylation of the Y394 by a phosphatase, which is likely to be either CD45 (D'Oro *et al.* 1996) or SHP-1 (Chiang & Sefton 2001). This implies that there will be a micro-environment enriched in activated Lck molecules in the vicinity of an engaged TCR. The overall activity of Lck kinase is thus likely to involve a dynamic balance between activation by Src kinases and inactivation by protein tyrosine phosphatases (PTP). Recent experiments suggest that the control of access to kinases and phosphatases in the T cell : APC contact zone may be by spatial reorganization of lipid rafts enriched in Src kinases but excluding CD45 and possibly other membrane-associated phosphatases (Van der Merwe *et al.* 2000).

Figure 1. Feedback control model. In the feedback control model, TCR engagement by peptide-MHC results in the recruitment of kinases as indicated by the labelled arrow (1). This alters the balance of the kinase-phosphatase 'futile cycle' and results in threshold activation due to feedback regulation for an agonist ligand. Positive feedback results from the action of activated kinase on enhancing further kinase activation. Negative feedback arises from activated kinase enhancing the activation of phosphatase, which then enhances the deactivation of kinase in a classical loop. Dashed arrows represent regulatory interactions. Activated enzymes are denoted by an asterisk.

Feedback effects are also important in the regulation of Lck activity. As noted above, Lck has a direct positive feedback effect on itself by *trans* auto-phosphorylation of the catalytic domain tyrosine residue (Yamaguchi & Hendrickson 1996). Significantly, auto-phosphorylation of Lck has been shown to increase nonlinearly with increasing concentrations (Wang *et al.* 1994). Negative feedback may also be important in the regulation of TCR activation, because the tyrosine phosphatase SHP-1 is activated by Lck and in turn dephosphorylates Lck at Y394 (Plas *et al.* 1996; Chiang & Sefton 2001). An appreciation of the system's nonlinear dynamical behaviour and the role of feedback is therefore likely to enhance our understanding of TCR activation (Germain & Štefanová 1999; Germain 2001).

We have drawn together recent developments in the signalling biology of the TCR to construct simple mathematical models for TCR activation, in which the degree of primed Lck recruitment to the TCR is proportional to the duration of ligand engagement. TCR activation depends on the balance between activated kinases and phosphatases, and this balance is regulated by feedback effects. The basic model assumes that both kinases and phosphatases have a low constitutive rate of activation. In addition, kinases have positive feedback from auto-phosphorylation, but also activate phosphatases which then inactivate them in a classical negative feedback loop, as illustrated in figure 1.

We have opted to extract the essential features of proximal TCR signalling that could provide functional insight rather than build a detailed mechanistic model. This is justified, as the main conclusions of the model are largely insensitive to the precise details of the model, and in particular more complicated models which take into account other kinases, phosphatases and feedback pathways documented in proximal TCR signalling also demonstrate the same qualitative behaviour (Chan 2001). Similarly, our results do not depend on the identification of Lck as the principal kinase involved and we fully expect them to hold

as a complete kinetic and biophysical description of the proximal signalling pathways is developed in the future.

2. MODELLING

We express the model shown in figure 1 as a set of ordinary differential equations to understand its dynamical behaviour. Let x and x^* be the local concentrations of inactive and active Lck near a TCR respectively, and $x_{\text{total}} = x + x^*$ be the total Lck concentration. Similarly, y , y^* and y_{total} are the equivalent local concentrations of phosphatase. We initially fix x_{total} and y_{total} and consider the kinetics for x^* and y^* . For each forward reaction, there is both a basal rate and a feedback rate catalysed by an enzyme. For simplicity, the backward reactions have not been split into basal and feedback rates as well, assuming that the enzyme catalysed rate dominates the basal rate. Each individual feedback pathway depends either linearly or nonlinearly on the concentration of the relevant activated enzyme. Mass action kinetics gives the following coupled equations:

$$\frac{dx^*}{dt} = k_1x + m_1f(x^*)x - n_1x^*y^* - d_1x^*, \quad (2.1)$$

$$\frac{dy^*}{dt} = k_2y + m_2x^*y - n_2y^* - d_2y^*, \quad (2.2)$$

where k_i are the basal forward rates, m_i are the catalysed forward rates, n_i are the backward rates, and d_i are the clearance rates (i.e. loss by diffusion and not available for further reactions).

In vitro data show that the positive feedback $f(x^*)$ for the auto-catalysis is nonlinear (Wang *et al.* 1994). Using least-squares fitting to fig. 5 in Wang *et al.* (1994) suggests that $f(x^*) = (x^*)^n$ where $1.5 \leq n \leq 2$. Bistability and the dynamical behaviour described in this paper can exist for both $n = 1.5$ and $n = 2$, and we have illustrated results for $f(x^*) = (x^*)^2$. Our simulations assume that the rate constants for catalysed reactions are much larger than uncatalysed reactions, but are otherwise arbitrary. We also assume that the rate of loss of active phosphatase activity is much slower relative to the rate of loss of Lck activity (Štefanová *et al.* 2003).

The stability and equilibrium states for x^* and y^* can be calculated using standard nonlinear dynamics tools. In particular, we are interested in understanding the changes in dynamical behaviour as x_{total} is varied. Bifurcation analysis shows that the threshold and bistable behaviour resulting from altering x_{total} are robust over a wide range of parameters.

For numerical simulations, we assume that ligand engagement results in a signal that recruits inactive Lck to the TCR at rate r . In the absence of ligand engagement, Lck is assumed to diffuse away at a linear rate d_0 . This leads to the following coupled equations:

$$\frac{dx}{dt} = r(l) + n_1x^*y^* - k_1x - m_1f(x^*)x - d_0x, \quad (2.3)$$

$$\frac{dx^*}{dt} = k_1x + m_1f(x^*)x - n_1x^*y^* - d_1x^*, \quad (2.4)$$

$$\frac{dy^*}{dt} = k_2y + m_2x^*y - n_2y^* - d_2y^*, \quad (2.5)$$

Figure 2. Threshold behaviour with Lck recruitment. Activation diagram showing equilibrium concentration of Lck* for various levels of recruited Lck. From left to right, as the total Lck accumulates, there comes a critical point (A) when positive feedback swamps the inhibition by phosphatase and Lck* levels suddenly increase sharply. This allows the TCR to act as a molecular switch which measures the recruitment of primed Lck and thus indirectly the duration of antigen engagement. Note that when moving in the reverse direction, the level of Lck* does not sharply drop at the same value of total Lck, but only when total Lck is much lower (B). This example of hysteresis gives the TCR a short-term memory of agonist binding. Parameter values: $k_1 = 0.01$, $k_2 = 0.01$, $m_1 = 1$, $m_2 = 1$, $n_1 = 1$, $n_2 = 0.02$, $d_1 = 0.15$, $d_2 = 0$, $x_{\text{total}} = \text{variable}$, $y_{\text{total}} = 1.2$.

where

$$r(l) = \begin{cases} \alpha & \text{if ligand bound} \\ 0 & \text{otherwise} \end{cases}$$

This implies that the availability of Lck is a saturable increasing function of the duration of ligand engagement, and Lck recruitment drives TCR activation.

We have also written stochastic simulations of the above rate equations to test the model when only small numbers of signalling molecules are present, using the exact and efficient algorithm first described by Gillespie (1977).

3. RESULTS

(a) All or none activation from Lck recruitment

Model analysis shows that as TCR engagement leads to a build-up of primed Lck, there is a critical concentration at which an abrupt change in behaviour occurs. Below this critical point, nearly all the Lck is in the primed but inactive form, but above it, nearly all the Lck is in the activated form (Lck*). We suggest that the time taken to recruit sufficient Lck to reach this critical concentration constitutes the TCR activation threshold. Of course, there may be more than one such threshold in the signalling pathway, given the diversity of protein tyrosine kinases and phosphatases known to interact directly with the TCR complex.

Figure 2 illustrates this behaviour, using a plot of the concentration of activated Lck against total Lck. When the concentration of Lck available to the TCR reaches a

Figure 3. Feedback control results in high specificity. (a) A simulation of a ligand that engages the TCR at time $t = 10$ s and dissociates at time $t = 24$ s. The figure assumes that the amount of recruited Lck is an increasing function of the duration of TCR ligand engagement for the reasons given in the text. It takes *ca.* 13 s of binding (at time $t = 23$ s) before the total Lck recruited crosses the threshold (point A in figure 2) and the level of Lck* suddenly rises and the TCR is activated. This sharp transition allows the TCR to clearly discriminate between ligands with different binding times. This is illustrated in (b), where a ligand that binds for only 7 s results in negligible Lck activation because the total Lck recruited fails to reach the threshold. Dashed line, total Lck; solid line, Lck*; dot and dashed line, phosphatase*; dotted line, signal. Parameter values used: $\alpha = 1$, $k_1 = 0.01$, $k_2 = 0.01$, $m_1 = 1$, $m_2 = 1$, $n_1 = 1$, $n_2 = 0.02$, $d_0 = 0.15$, $d_1 = 0.15$, $d_2 = 0$, $x_{\text{total}} = \text{variable}$, $y_{\text{total}} = 1.2$.

certain threshold, there will be a sudden jump, to a state where most of the Lck molecules are activated. Below this threshold, nearly all the Lck present are in an inactive state. If Lck recruitment is directly related to the duration of ligand engagement, this suggests that ligand engagement for longer than a critical period will result in TCR activation, which is consistent with experiments showing that the dissociation time is the best predictor of T-cell response (Davis *et al.* 1998).

Intuitively, this is easy to understand. Initially, the concentration of Lck is low and the surfeit of phosphatases results in the rapid dephosphorylation of any activated Lck, hence inactivating it. However, when the TCR is bound, Lck is recruited until it achieves a critical mass when enough activated Lck is present to phosphorylate other Lck molecules before the phosphatase can act. Positive feedback then ensures that the system rapidly converges to a state where nearly all the Lck is in an active state.

(b) *Threshold activation results in specificity of ligand discrimination*

Because of the presence of a threshold for TCR activation, ligands that bind for only slightly different durations can be distinguished. For example, if it takes 10 s to recruit sufficient Lck to cross the threshold, then a ligand which binds for (say) 8 s will result in negligible TCR activation, whereas one that binds for 12 s will result in significant TCR activation. This allows the TCR to discriminate between ligands with only small differences in dissociation times, i.e. it gives the T cell high specificity. Figure 3 shows the dramatic difference in TCR activation between ligands that differ in binding time by only twofold.

As with the original kinetic proofreading model, discrimination between 'good' and 'bad' ligands depends mainly on the ligand's k_{diss} and is essentially independent of the concentration. This is because by having a set

activation threshold, the receptor is effectively measuring the occupancy time of the ligand, which is determined by the k_{diss} . Given an activation threshold of τ s, the fraction of ligand that binds long enough to cross this threshold and thus activate the TCR is given by $e^{-k_{\text{diss}}\tau}$. For example, because $t_{1/2} = \log(2)/k_{\text{diss}}$, a 'good' ligand that has a $k_{\text{diss}} = \log(2)/\tau$ will activate 50% of the TCRs it is bound to. By contrast, a 'poor' ligand that has a k_{diss} 100-fold larger will activate only $7.89 \times 10^{-29}\%$ of the TCRs it is bound to. However, as with the kinetic proofreading model, very high concentrations of ligands which differ only a fewfold in k_{diss} can activate the TCR because of stochastic dissociation. This highlights the need for other mechanisms, such as receptor cross-talk, to ensure specificity at the cellular level (Chan *et al.* 2001).

(c) *Inhibition by antagonist ligands*

Because Lck both activates and recruits the tyrosine phosphatase SHP-1, the model predicts that SHP-1 levels will be higher after ligand engagement by both antagonist and agonist ligands. This leads to a relative refractory period during which subsequent engagement by the same TCR with an agonist will fail to trigger TCR activation (figure 4). In addition, the recruited SHP-1 may also inhibit neighbouring TCRs, which could explain the ability of small numbers of antagonist ligand to inhibit T-cell activation by agonist ligands present in excess. This would not happen for agonist ligands because the activity of SHP-1 is blocked by the production of ERK-1 (Štefanová *et al.* 2003). This spreading inhibition and protection significantly improves the specificity of T-cell activation (Germain & Štefanová 1999; Chan *et al.* 2001).

(d) *Hysteresis and rebinding*

The feedback model predicts that once the TCR is activated by a ligand, it will remain in the active state for a short time even after the ligand has dissociated. This

Figure 4. Phosphatase recruitment by an antagonist ligand results in a refractory period. (a) An antagonist that binds for 7 s does not trigger TCR activation, but leads to an increased level of activated phosphatase. This prevents a subsequent agonist ligand that binds for 14 s from activating the TCR. (b) A null ligand that binds for 2 s does not result in significant phosphatase activation, and subsequent binding by an agonist ligand is unaffected. Dashed line, total Lck; solid line, Lck*; dot and dashed line, phosphatase*; dotted line, signal. Parameter values as in figure 3.

Figure 5. Rebinding and hysteresis can sustain signalling. (a) Once a TCR is activated by an agonist ligand (which binds for 14 s), signalling can be sustained by serial encounters with poor ligands that bind for only 2 s each. Such an effect might be important in sustaining signalling at the immune synapse, where the time between ligand encounters is reduced as a result of enforced proximity. (b) shows that this is also a safe mechanism for signal amplification and maintenance, because it requires the presence of an agonist ligand in the first place to 'jump start' the reaction. A medium-affinity ligand that binds for 7 s does not activate the TCR, even when it is followed by the same set of serial encounters with poor ligands. Dashed line, total Lck; solid line, Lck*; dot and dashed line, phosphatase*; dotted line, signal. Parameter values as in figure 3.

occurs because the threshold for activation (where nearly all the $Lck \rightarrow Lck^*$) is higher than the threshold for deactivation (where nearly all the $Lck^* \rightarrow Lck$), a phenomenon known as hysteresis (illustrated in figure 2). The activated TCR can thus be maintained in an activated state even after the original agonist ligand dissociates, by successively rebinding to a series of ligands which would otherwise not be able to activate the TCR on their own (figure 5). The length of this transition period where the TCR can be re-stimulated by non-specific ligands depends on the time it takes for the recruited Lck to be lost, as well as the strength of negative feedback.

The hysteresis effect has very interesting implications for the role of self peptide-MHC complexes, which have some affinity for the TCR owing to positive selection. If the binding of self-ligands is sufficiently rapid, they may

be able to maintain the activation state of a TCR initially triggered by an agonist ligand even though the duration of engagement is short, thus sustaining signalling even after the original ligand has dissociated. This process for sustained signalling by self-ligands is unlikely to result in autoimmunity, because the initial activation must always be by a ligand with a long binding time (see figure 5).

(e) Stochastic modelling

The previous discussion describes the model in the context of an ordinary differential equations (ODE) model. However, the ODE model is valid for only large numbers of molecules and ignores stochastic fluctuations. Because the actual numbers of signalling molecules in the neighbourhood of a TCR are likely to be small, stochastic fluctuations may significantly impact the system behaviour.

Figure 6. Stochastic simulations of the feedback control model using Gillespie's algorithm. (a) Frequency distribution of activation times from 100 000 simulations (crosses). The mean activation time is 15.74 with a standard deviation of 5.00, and the mean number of Lck molecules present at activation is 14.6. The maximum likelihood fit to a gamma distribution is also shown (dashed line). (b) A sample run showing the abrupt increase in number of Lck* molecules upon activation. Note that the variables x and y given below now refer to actual numbers of Lck and phosphatase molecules respectively, and not concentrations. Parameter values: $\alpha = 1$, $k_1 = 0.15$, $k_2 = 0.01$, $m_1 = 0.1$, $m_2 = 1$, $n_1 = 0.2$, $n_2 = 0.02$, $d_0 = 0.01$, $d_1 = 0.01$, $d_2 = 0$, $x_{\text{total}} = \text{variable}$, $y_{\text{total}} = 10$.

We have therefore run stochastic versions of the model using Gillespie's algorithm (Gillespie 1977) to study the behaviour of the system when there are small numbers of molecules involved.

Figure 6a shows the frequency distribution of activation times for 100 000 simulations with 10 phosphatase molecules and where the number of Lck molecules recruited increases linearly with time in a stochastic manner. We consider a TCR to be activated if there are 10 or more activated Lck molecules present. Although the step function predicted by the differential model is lost, as would be expected, there is still a high degree of specificity as shown by the steepness of the response slope. Using a maximum-likelihood fit of the cumulative response to a gamma distribution, we can calculate the amplification factor for a ligand that binds for the mean activation time compared with one that binds for a 10-fold shorter period is *ca.* 2.07×10^7 . Therefore, it is clear that the feedback control model has a similar ability to discriminate ligands with high specificity even when there are only small numbers of signalling molecules present. A representative run of the stochastic model showing the threshold response is shown in figure 6b.

However, the mean number of recruited Lck molecules to the TCR before activation occurs affects the specificity of the response. For example, shortening the onset of the activation threshold by increasing the rate of auto-catalysis m_1 decreases the specificity. With increasing values of m_1 , the mean activation time t_{act} is decreased, and fewer Lck molecules n need to be recruited on average to trigger TCR activation. However, the ratio of variance to mean value also increases. When fitted to a gamma distribution, this is reflected in progressively decreasing amplification ratios for a ligand binding for the mean activation time compared with one that binds for a 10-fold shorter duration (table 1).

We have previously shown that optimal kinetic proofreading requires that all the modification rates k_p are identical (Chan *et al.* 2003). For a single TCR, this is

equivalent to having identical probabilities λ of transiting from one intermediate state C_{i-1} to C_i per unit time. Under these conditions, the activation times of individual TCRs with kinetic proofreading follow a gamma distribution with the shape parameter determined by the length of the chain N , and the rate parameter determined by λ (Chan *et al.* 2003). For example, the fit to a gamma distribution shown in figure 6a implies that the feedback model with the given parameters offers the same specificity as a kinetic proofreading chain with $12 < N < 13$ and probability of transition from one intermediate state to the next of *ca.* 1.41 s^{-1} . (cf. fig. 8 in Chan *et al.* (2003) for kinetic proofreading).

4. DISCUSSION

In this paper, we suggest that kinase-phosphatase 'futile cycles' interacting dynamically by both positive and negative feedback loops result in a triggering threshold and hysteresis, providing a simple and robust mechanism for TCR specificity and ligand discrimination. The role of tyrosine auto-phosphorylation and threshold behaviour has been previously modelled at the level of the T cell to explain the persistence of anergy in helper T cells (Kaufman *et al.* 1996), but our model proposes that these futile cycles are involved in *individual* TCR recognition events. Although we have focused on the role of Lck recruitment, the mirror model where phosphatase depletion upon ligand engagement (or both processes occur simultaneously) is the major factor driving TCR activation gives similar qualitative results (not shown).

Our model suggests an alternative mechanism for kinetic proofreading using local accumulation of signalling molecules to provide specificity rather than a linear chain of modifications. One assumption of the model is that dynamic interactions of kinase and phosphatase molecules in the local neighbourhood of the TCR are necessary to provide a threshold for activation. This does not contradict the current view of TCR signalling involving assembly

Table 1. Statistics for stochastic activation of the feedback control model for different values of m_1 .

(Statistics were obtained from 100 000 runs for each set of parameter values. The time of activation is indicated by t_{act} and n is the total number of Lck molecules at activation. The amplification factor is the ratio of number of TCRs activated by a ligand with $k_{\text{diss}} = \log(2)/\tau$ compared with one with k_{diss} 10-fold smaller, where τ is the activation threshold. This is calculated from the fit to a gamma distribution as discussed in the text and is a measure of TCR specificity. The column labelled N indicates the number of steps in an ideal kinetic proofreading chain that would be necessary to achieve the same amplification factor (Chan *et al.* 2003).)

m_1	standard deviation		mean n	standard deviation		amplification factor	N
	mean t_{act}	of t_{act}		of n			
0.05	17.73	4.98	16.30	2.69	2.05×10^8	12–13	
0.10	15.74	5.00	14.60	2.94	2.07×10^7	11–12	
0.15	14.57	4.92	13.59	2.95	0.28×10^6	10–11	
0.20	13.80	4.82	12.92	2.88	5.91×10^6	10–11	
0.25	13.26	4.71	12.45	2.78	4.83×10^6	9–10	

of a complex signalling scaffold, because the known adaptor proteins involved in TCR scaffolding are downstream of ZAP-70 activation (Burack *et al.* 2002) and therefore unlikely to be directly involved in ligand discrimination. Mobile interactions are probably also important for membrane-associated ZAP-70, which has been observed during T-cell activation to continually associate and dissociate from TCR signalling complexes within seconds (Sloan-Lancaster *et al.* 1998; Bunnell *et al.* 2002). A related assumption is that the 'stoichiometry' of Lck to TCR is several to one, at least during the process of activation. This is only problematic if Lck only associates with CD4/CD8 and formation of a stable coreceptor:TCR complex is critical for receptor activation. As discussed in § 1, there is evidence that non-coreceptor associated membrane Lck also has an important role in the initiation of TCR activation. In addition, the interaction of CD4/CD8 with the TCR:MHC complex is characterized by extremely fast dissociation (König 2002), suggesting a more dynamical interaction. This is supported by recent experiments showing that CD4 is not necessarily co-localized with the TCR or Lck during T-cell activation (Holdorf *et al.* 2002; Wülfing *et al.* 2002). With the rapid advances in real-time imaging of signalling molecules, these assumptions should soon be capable of being directly tested.

The model also ignores the potential role of diffusion of signalling molecules between neighbouring TCRs. However, the critical period for ligand discrimination and TCR signalling occurs before the clustering of TCRs in the immune synapse (Lee, K.-H. *et al.* 2002). During this period, TCRs are presumably well separated and the effects of signalling molecule diffusion from one receptor to another would be expected to be minimal.

It has been suggested that TCR activation threshold tuning may occur adaptively, allowing the T cell to set a threshold to optimally filter out self peptide-MHC complexes and detect foreign antigens (Grossman & Paul 1992). Our model suggests a simple mechanism for such threshold tuning by altering phosphatase levels. Experimentally, tuning of T-cell reactivity has been detected both in the thymus (Wong *et al.* 2001) and in the periphery (Smith *et al.* 2001), and appears to involve the regulated expression of CD5, which associates with the tyrosine phosphatase SHP-1 (Perez-Villar *et al.* 1999).

This will alter the kinase-phosphatase balance and hence the TCR activation threshold.

The hysteresis effect shown in these models gives the TCR a short-term memory of agonist binding, allowing subsequent rebinding by the same or another ligand to influence the TCR signal. This introduces the possibility that self peptide-MHC complexes may play a role in sustaining signalling at the immune synapse or possibly even earlier, during the formation of small TCR clusters before their coalescence to form the immune synapse. This effect may also contribute to the maintenance of sustained signalling over several hours necessary for naive T-cell activation (Iezzi *et al.* 1998). We also believe that the model provides a simple mechanism explaining how endogenous 'null' peptide-MHC complexes can contribute to T-cell signalling in the presence of small amounts of agonist peptide-MHC, as recently demonstrated experimentally (Wülfing *et al.* 2002).

An alternative proposal for the effect of short-binding peptide-MHC complexes in enhancing the signal from limiting amounts of agonist ligands is 'stochastic resonance' (Germain & Štefanová 1999; Wülfing *et al.* 2002). Stochastic resonance requires some periodicity or coherence of the primary signal (Gammaitoni *et al.* 1998). However, the signals generated by the TCR in response to the presence of an agonist ligand will be highly irregular and essentially random, and so the addition of noise is unlikely to increase sensitivity by this mechanism.

Using a mathematical model of synapse assembly, Lee, K.-H. *et al.* (2002) have proposed that null or self-peptides can facilitate the assembly of an immune synapse and thus contribute to enhanced signalling. Such ligands are required to have a high k_{on} in combination with a low k_{off} such that the K_D is roughly constant. Our model differs in suggesting that such ligands can contribute to signalling at the level of individual TCRs. Experimentally, the models can be distinguished by testing whether 'null' ligands can positively contribute to T-cell signalling prior to the formation of the mature immune synapse. A possible test would be whether tyrosine kinase activity can be enhanced with 'null' ligands, because tyrosine kinase activity mostly precedes formation of the mature immune synapse (Lee S.-J. E *et al.* 2002).

Our modelling shows that the properties of threshold activation and hysteresis might be important for TCR

ligand discrimination. In fact, we propose that the purpose of positive selection may not be to discard 'useless' thymocytes, but to select for TCRs able to bind at least minimally to self-ligands and hence to sustain and amplify signalling. We describe here a possible experiment to test this proposal. Antigen-specific T cells are obtained from immunized animals in which positive selection happened on a single peptide A or B (for example, using covalent class II MHC-peptide complexes). We now compare the augmentation of signalling in the experimental protocol described by Wülfing *et al.* (2002) to the immunizing peptide using either the selecting 'irrelevant' peptide A or B as 'background'. T cells selected on a background of peptide A would be expected to exhibit greater signal enhancement in the presence of peptide A over peptide B. Conversely, T cells selected on a background of peptide B would be expected to show the reverse result.

Negative regulation of receptor protein kinases (RTKs) by PTPs is widely documented (Östman & Böhmer 2001), and it is widely believed that their main role is to down-modulate signalling by the RTKs. We suggest that such circuits may commonly form bistable switching circuits, with more subtle roles for PTPs in tuning switching behaviour, as shown in our model for TCR activation. This would expand the range of cellular systems known or postulated to have bistable behaviour (Ferrell 2002).

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REFERENCES

- Abraham, N., Miceli, M. C., Parnes, J. R. & Veillette, A. 1991 Enhancement of T-cell responsiveness by the lymphocyte-specific tyrosine protein kinase p56^{lck}. *Nature* **350**, 62–66.
- Ardouin, L. (and 10 others) 1999 Crippling of CD3- ζ ITAMs does not impair T cell receptor signalling. *Immunity* **10**, 409–420.
- Bonnard, M., Maroun, C. R. & Julius, M. 1997 Physical association of CD4 and CD45 in primary, resting CD4⁺ T cells. *Cell. Immunol.* **175**, 1–11.
- Bunnell, S. C., Hong, D. I., Kardon, J. R., Yamazaki, T., McGlade, C. J., Barr, V. A. & Samelson, L. E. 2002 T-cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *J. Cell Biol.* **158**, 1263–1275.
- Burack, W. R., Cheng, A. M. & Shaw, A. S. 2002 Scaffolds, adaptors and linkers of TCR signaling: theory and practice. *Curr. Opin. Immunol.* **14**, 312–316.
- Burkhardt, A. L., Stealey, B., Rowley, R. B., Mahajan, S., Prendergast, M., Fargnoli, J. & Bolen, J. B. 1994 Temporal regulation of non-transmembrane protein tyrosine kinase enzyme activity following T cell antigen receptor engagement. *J. Biol. Chem.* **269**, 23 642–23 647.
- Chan, C., George, A. J. T. & Stark, J. 2001 Cooperative enhancement of T cell specificity in a lattice. *Proc. Natl Acad. Sci. USA* **98**, 5758–5763.
- Chan, C., George, A. J. T. & Stark, J. 2003 T cell sensitivity and specificity: kinetic proofreading revisited. *Discrete Continuous Dynam. Systems B* **3**, 343–360.
- Chan, C. C. W. 2001 Modelling T cell activation. PhD thesis, University College London.
- Chiang, G. G. & Sefton, B. M. 2001 Specific dephosphorylation of the Lck tyrosine protein kinase at Tyr-394 by the SHP-1 protein-tyrosine phosphatase. *J. Biol. Chem.* **276**, 23 173–23 178.
- Davis, M. M., Boniface, J. J., Reich, Z., Lyons, D., Hampl, J., Arden, B. & Chien, Y. 1998 Ligand recognition by $\alpha\beta$ T cell receptors. *A. Rev. Immunol.* **16**, 523–544.
- D'Oro, U., Sakaguchi, K., Appella, E. & Ashwell, J. D. 1996 Mutational analysis of Lck in CD45-negative T cells: dominant role of tyrosine 394 phosphorylation in kinase activity. *Mol. Cell Biol.* **16**, 4996–5003.
- Ehrlich, L. I. R., Ebert, P. J., Krummel, M. F., Weiss, A. & Davis, M. M. 2002 Dynamics of p56lck translocation to the T cell immunological synapse following agonist and antagonist stimulation. *Immunity* **17**, 809–822.
- Ferrell Jr, J. E. 2002 Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. *Curr. Biol.* **6**, 140–148.
- Gammaitoni, L., Hanggi, P., Jung, P. & Marchesoni, F. 1998 Stochastic resonance. *Rev. Mod. Phys.* **70**, 223–287.
- Germain, R. N. 2001 The art of the probable: system control in the adaptive immune system. *Science* **293**, 240–245.
- Germain, R. N. & Štefanová, I. 1999 The dynamics of T cell receptor signalling: complex orchestration and the key roles of tempo and cooperation. *A. Rev. Immunol.* **17**, 467–522.
- Gil, D., Schamel, W. W., Montoya, M., Sánchez-Madrid, F. & Alarcón, B. 2002 Recruitment of Nek by CD3 ϵ reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. *Cell* **109**, 901–912.
- Gillespie, D. T. 1977 Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.* **81**, 2340–2361.
- Grossman, Z. & Paul, W. E. 1992 Adaptive cellular interactions in the immune system: the tunable activation threshold and the significance of subthreshold responses. *Proc. Natl Acad. Sci. USA* **89**, 10 365–10 369.
- Holdorf, A. D., Burack, R., Allen, P. M. & Shaw, A. S. 2002 Regulation of Lck activity by CD4 and CD28 in the immunological synapse. *Nature Immunol.* **3**, 259–263.
- Iezzi, G., Karjalainen, K. & Lanzavecchia, A. 1998 The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* **8**, 89–95.
- Kaufman, M., Andris, F. & Leo, O. 1996 A model for antigen induced T cell unresponsiveness based on autophosphorylative protein tyrosine kinase activity. *Int. Immunol.* **8**, 613–624.
- Kersh, G. J., Kersh, E. N., Fremont, D. H. & Allen, P. M. 1998 High- and low-potency ligands with similar affinities for the TCR: The importance of kinetics in TCR signaling. *Immunity* **9**, 817–826.
- König, R. 2002 Interactions between MHC molecules and co-receptors of the TCR. *Curr. Opin. Immunol.* **14**, 75–83.
- Lee, K.-H., Holdorf, A. D., Dustin, M. L., Chan, A. C., Allen, P. M. & Shaw, A. S. 2002 T cell receptor signaling precedes immunological synapse formation. *Science* **295**, 1539–1542.
- Lee, S.-J. E., Hori, Y., Groves, J. T., Dustin, M. L. & Chakraborty, A. K. 2002 Correlation of a dynamic model for immunological synapse formation with effector functions: two pathways to synapse formation. *Trends Immunol.* **23**, 492–499.
- McFarland, E. D. C., Hurley, T. R., Pingel, J. T., Sefton, B. M., Shaw, A. & Thomas, M. L. 1993 Correlation between Src family member regulation by the protein tyrosine phosphatase CD45 and transmembrane signaling through the T-cell receptor. *Proc. Natl Acad. Sci. USA* **90**, 1402–1406.
- McKeithan, T. W. 1995 Kinetic proofreading in T-cell receptor signal transduction. *Proc. Natl Acad. Sci. USA* **92**, 5042–5046.
- Madrenas, J., Wange, R. L., Wang, J. L., Isakov, N., Samelson, L. E. & Germain, R. N. 1995 ζ phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. *Science* **267**, 515–518.

- Molina, T. J. (and 11 others) 1992 Profound block in thymocyte development in mice lacking p56^{lck}. *Nature* **357**, 161–164.
- Ostergaard, H. L., Shackelford, D. A., Hurley, T. R., Johnson, P., Hyman, R., Sefton, B. M. & Trowbridge, I. S. 1989 Expression of CD45 alters phosphorylation of the lck-encoded tyrosine protein kinase in murine lymphoma-T cell lines. *Proc. Natl Acad. Sci. USA* **86**, 8959–8963.
- Östman, A. & Böhmer, F.-D. 2001 Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. *Trends Cell Biol.* **11**, 258–266.
- Perez-Villar, J. J., Whitney, G. S., Bowen, M. A., Hewgill, D. H., Arullo, A. A. & Kanner, S. B. 1999 CD5 negatively regulates the T-cell antigen receptor signal transduction pathway: involvement of SH2-containing phosphotyrosine phosphatase SHP-1. *Mol. Cell Biol.* **19**, 2903–2912.
- Plas, D. R., Johnson, R., Pingel, J. T., Matthews, R., Dalton, M., Roy, G., Chan, A. C. & Thomas, M. L. 1996 Direct regulation of ZAP-70 by SHP-1 in T cell antigen receptor signaling. *Science* **272**, 1173–1176.
- Sicheri, F. & Kuriyan, J. 1997 Structures of Src-family tyrosine kinases. *Curr. Opin. Struct. Biol.* **7**, 777–785.
- Sloan-Lancaster, J., Shaw, A. S., Rothbard, J. B. & Allen, P. M. 1994 Partial T cell signaling: altered phospho- ζ and lack of ZAP-70 recruitment in APL-induced T cell anergy. *Cell* **79**, 913–922.
- Sloan-Lancaster, J., Presley, J., Ellenberg, J., Yamazaki, T., Lippincott-Schwartz, J. & Samelson, L. E. 1998 ZAP-70 association with T cell receptor (TCR): fluorescence imaging of dynamic changes upon cellular stimulation. *J. Cell Biol.* **143**, 613–624.
- Smith, K., Seddon, B., Purbhoo, M. A., Zamoyska, R., Fisher, A. G. & Merckenschlager, M. 2001 Sensory adaptation in naive peripheral CD 4 T cells. *J. Exp. Med.* **194**, 1253–1261.
- Štefanová, I., Hemmer, B., Vergelli, M., Martin, R., Biddison, W. E. & Germain, R. N. 2003 TCR ligand discrimination is enforced by competing ERK positive and SHP-1 negative feedback pathways. *Nature Immunol.* **4**, 248–254.
- Stein, P. L., Lee, H. M., Rich, S. & Soriano, P. 1992 pp59^{lyn} mutant mice display differential signaling in thymocytes and peripheral T cells. *Cell* **70**, 741–750.
- Sykulev, Y., Joo, M., Vturina, I., Tsomides, T. J. & Eisen, H. N. 1996 Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity* **4**, 565–571.
- Thome, M., Duplay, P., Guttinger, M. & Acuto, O. 1995 Syk and ZAP-70 mediate recruitment of p56^{lck}/CD4 to the activated T cell receptor/CD3/ ζ complex. *J. Exp. Med.* **181**, 1997–2006.
- Thome, M., Germain, V., DiSanto, J. P. & Acuto, O. 1996 The p56^{lck} SH2 domain mediates recruitment of CD8/p56^{lck} to the activated T cell receptor/CD3/ ζ complex. *Eur. J. Immunol.* **26**, 2093–2100.
- Van der Merwe, P. A., Davis, S. J., Shaw, A. S. & Dustin, M. L. 2000 Cytoskeletal polarization and redistribution of cell-surface molecules during T cell antigen recognition. *Semin. Immunol.* **12**, 5–21.
- Wang, S., Lehr, R., Stevis, P., Wang, X.-M., Trevillyan, J. & Faltynek, C. R. 1994 p56lck interactions accelerate autophosphorylation and enhance activity toward exogenous substrates. *Arch. Biochem. Biophys.* **315**, 60–67.
- Wong, P., Barton, G. M., Forbush, K. A. & Rudensky, A. Y. 2001 Dynamic tuning of T cell reactivity by self-peptide-major histocompatibility complex ligands. *J. Exp. Med.* **193**, 1179–1187.
- Wülfing, C., Sumen, C., Sjaastad, M. D., Wu, L. C., Dustin, M. L. & Davis, M. M. 2002 Costimulation and endogenous MHC ligands contribute to T cell recognition. *Nature Immunol.* **3**, 42–47.
- Yamaguchi, H. & Hendrickson, W. A. 1996 Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. *Nature* **384**, 484–489.

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