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A conformation- and avidity-based proofreading mechanism for the TCR–CD3 complex

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During antigen recognition, T cells show high sensitivity and specificity, and a wide dynamic range. Paradoxically, these characteristics are based on low-affinity receptor–ligand interactions [between the T-cell antigen receptor (TCR–CD3) complex and the antigen peptide bound to MHC]. Recent evidence indicates that the TCR–CD3 is expressed as multivalent complexes in the membrane of non-stimulated T cells and that conformational changes in the TCR–CD3 can be induced by strong but not weak agonists. Here, we propose a thermodynamic model whereby the specificity of the TCR–CD3–pMHC interaction is explained by its multivalent nature. We also propose that the free energy barriers involved in the change in conformation of the receptor impose a response threshold and determine the kinetic properties of recognition. Finally, we suggest that multivalent TCR–CD3s can amplify signals by spreading them from pMHC-engaged TCR–CD3s to unengaged complexes as a consequence of the cooperativity in the system.

Introduction

Although the subunit composition of the T-cell antigen receptor (TCR–CD3) complex (referred to as TCR–CD3) was defined nearly two decades ago, its stoichiometry remains unclear [1,2]. Using biochemical and electron microscopy data from fixed intact T cells, we recently proposed that the TCR–CD3 is expressed in the plasma membrane as a mixture of complexes of different sizes [3]. These results, together with data from previous co-precipitation [4], FRET [4] and density centrifugation [5] studies, strongly indicate that the TCR–CD3 is not exclusively expressed at the cell surface as a monovalent complex with only a single TCRαβ heterodimer. Rather, the 'entirely monovalent' stoichiometry is merely an artifact of receptor solubilization in the detergent used, digitonin. The preformed multivalent TCR–CD3s are too small to be seen by confocal microscopy, because they usually contain between two and ten individual TCR–CD3 units (20–100 nm in diameter) and only rarely have a maximum of 20 units [3]. Hence, these complexes are different from the so-called TCR–CD3 patches or microclusters that are detected by light microscopy following TCR–CD3 stimulation and subsequent receptor aggregation [6–8]. The TCR–CD3 microclusters are longer, having an average size of 50 TCR–CD3 units. The pre-existing multivalent TCR–CD3s of up to 20 units could alternatively be referred to as nanoclusters.

The multivalent arrangement of the TCR–CD3 might be necessary for this receptor to fulfill a given function. Indeed, when compared with other membrane receptors, TCR–CD3 signaling is particularly intriguing. The receptor must first distinguish as few as ten antigen-peptide-ligand–MHC (pMHC) complexes [9,10] against a background of hundreds of thousands of closely related potential ligands (self peptides–MHC). This is achieved through a low-affinity interaction (0.1–50 μM), the result of fast dissociation and slow association rates [11–13]. Furthermore, T cells have a wide dynamic range that enables them to mount an escalated response to pMHC concentrations that differ by at least four-to-six orders of magnitude [3,14,15].

Several mechanisms have been proposed to resolve the paradox of high sensitivity and low affinity. The collaboration of coreceptors in MHC recognition helps to lower the threshold for activation but does not influence specificity [16]. The existence of multiple signaling cassettes that contain tyrosine- and leucine-rich motifs (immunoreceptor tyrosine-based activation motifs, ITAMs) in the cytoplasmic tails of the TCR–CD3 subunits also helps to amplify the activation signal. Nevertheless, there is little information to explain how the initial TCR–CD3–pMHC interaction activates tyrosine phosphorylation.

Here, we hypothesize that the multivalent nature of the TCR–CD3 could explain its high selectivity, high sensitivity and wide dynamic range, and that this could also be necessary for initiation of TCR-CD3 signaling. We discuss recent data regarding conformational changes in the TCR–CD3 and propose a model for ligand recognition and signal transmission. In our model, the conformational

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change triggered in the TCR–CD3 forms part of a proofreading mechanism that translates intrinsic affinity into productive TCR–CD3 signaling, thereby enabling T cells to sense the quality of the ligand. Furthermore, the large TCR–CD3 assemblies might serve to amplify the initial triggering event by propagating the conformational change to the TCR–CD3 engaged with self peptide–MHC ligands within the same cluster.

Ligand binding transmits a conformational change, conveying information on the quality of the pMHC

Two models have been proposed to account for the exquisite capacity of T cells to discriminate between pMHC antigens. The first, a kinetic proofreading model [17], proposes that the signaling machinery of T cells imposes activation thresholds based on the time of occupancy of the TCR–CD3 cluster [17,18]. Although the kinetic proofreading model also considers events such as clustering [17], most groups referring to this model consider the discrimination of agonist pMHCs and non-agonist pMHCs as dependent only on the quantity of a given signal, and not on its quality. According to this model, the molecular sieve responsible for discrimination is intracellular, and a recent modification of this model has proposed that positive and negative intracellular feedback loops combine to fine-tune the response to different pMHCs [19].

Thus, the quantitative models rely on the half-life of the TCR–pMHC interaction, which in general is well correlated with the strength of the ligand [11–13]. However, there are numerous exceptions: strong pMHC ligands with similar or shorter half-lives than weak ligands [20,21]. Three of these outliers were shown to promote large changes in heat capacity and were indicative of conformational changes that correlated with the induced fit of the pMHC-binding complementarity-determining-region (CDR) loops in the TCRαβ heterodimer [22].

The second model of pMHC discrimination is based on the idea that the agonist pMHC induces a specific conformational change in the TCR–CD3 cluster that non-agonist pMHC ligands cannot produce. Therefore, in contrast to the kinetic proofreading model, this model is based on the quality of the signal that emanates from the TCR–CD3 rather than the quantity of the intracellular signals. Indeed, when we examine the whole TCR–CD3 as the subject of the conformational change and not just the extracellular domains (ectodomains) of the TCRαβ heterodimer used for X-ray crystallography [22,23], we find that it undergoes a conformational change that is induced on binding to agonist pMHC but not to non-agonist pMHC [24]. This rearrangement in the structure of the TCR–CD3 exposes a proline-rich sequence (PRS) in the cytoplasmic tail of CD3ε [25,26]; the rearrangement was detected in the presence of the Src kinase inhibitor PP2, and following stimulation at 0°C, and it was also demonstrated in a cell-free, purified TCR–CD3. Therefore, exposure of the PRS in CD3ε was not caused by downstream TCR–CD3 signaling effects such as ITAM phosphorylation but rather it was a change in TCR–CD3 conformation induced directly by ligand binding.

Exposure of the PRS was originally demonstrated in a pull-down assay in which the engaged TCR–CD3 was shown to bind to the SH3.1 domain of the adaptor protein Nck [25]. More recently, the monoclonal antibody APA1/1, which specifically recognizes the PRS of CD3ε, was used to detect the exposure of the PRS upon TCR–CD3 ligation [24]. The epitope recognized by APA1/1 is displayed even if the T cells are stimulated at 0°C or in the presence of PP2. Hence, the exposure of the PRS appears to be intrinsic to the TCR–CD3 and independent of enzymatic activity.

Unlike the Nck pull-down assay, APA1/1 staining is useful to demonstrate the presence of T cells whose TCR–CD3 has undergone this conformational change in culture and in fixed tissues. In this regard, partial agonist peptides have been shown to induce the formation of abnormal immune synapses [27–30]. Using APA1/1 staining, we have shown that TCR–CD3s undergo the conformational change in immune synapses formed by strong agonists but not by partial agonists [24]. Our data therefore support the hypothesis that the conformational change in the TCR–CD3 underlies ligand discrimination.

An avidity proofreading model coupled to a conformational change in the TCR–CD3 as a readout of productive engagement

The effect of forming multivalent TCR–CD3s on ligand discrimination is most evident when the avidity of pMHC ligand recognition is considered. MHC class II molecules have been shown to form dimers [31,32] or even clusters [33], and a subset of MHC class II molecules has been localized in specific membrane microdomains rich in tetraspanin family proteins [34]. Furthermore, this 'tetraspan' subset of MHC class II has been suggested to have a decisive role in activating T cells at reduced concentrations of pMHC, and even to concentrate a selected set of peptide antigens [33]. If we assume the existence of similar clusters for MHC class I, we could be faced with a highly specific mechanism for ligand recognition that makes use of the interactions between multivalent TCR–CD3s and multivalent pMHC oligomers.

This mechanism is based on two processes that act coordinately. First, the different affinities of the TCR–CD3 for agonist and non-agonist pMHC are translated into different residence times (kinetic proofreading) [17]. The difference in residence times increases exponentially with multivalency (Box 1). Second, multivalent binding enables the TCR–CD3 to discriminate between agonists that induce a conformational change in the receptor and non-agonists that do not. The conformational change increases the avidity of the interaction, which is defined in the model as the apparent binding affinity (K_A^{app}; see the online supplementary material). When a MHC oligomer is loaded with two or more agonist peptides, the TCR–CD3 oligomers can oscillate between the monovalent and bivalent (or multivalent) engagement states, depending on the affinity of the interaction. This equilibrium might constitute a proofreading mechanism for the antigen quality if only multivalent engagement of the TCR–CD3 leads to the fully active state of the complex (Figure 1).

Box 1. Consequences of multivalent binding: a ligand-receptor model

Here, inspired by recent proposals [3,33,53], we explore the consequences of multivalent binding and formulate a theoretical model of ligand–receptor interactions that explicitly takes into account ligand and receptor multivalency. This model (Figure I), demonstrates explicitly the qualitative consequences that clustering and the conformational change have on ligand selectivity. For simplicity, we only consider situations at equilibrium, and we have neglected important kinetic aspects of the interaction that are known to be relevant to distinguishing the quality of the ligand. For example, the half-life of the TCR–CD3–pMHC interaction measured through the dissociation constant (K_{off}) might have an important influence on the time during which the conformational change in the TCR–CD3 is exposed.

In our model, we consider a cluster of TCR–CD3 complexes formed by a large, effectively infinite, number of TCR–CD3 units binding to a population of MHC molecules. Each MHC ligand itself consists of a multivalent cluster of presenting sites (v). Each peptide in the MHC cluster binds to a single site in a different TCR–CD3 unit within the receptor cluster. The model for the resulting binding process is illustrated in Figure I. We assume that each agonist peptide within the MHC molecule has an identical intrinsic affinity, K_A, for each site in the TCR cluster, which is taken as an average of the individual apparent affinities of the peptides. In addition, the binding of two or more peptides from the MHC to the TCR–CD3 cluster introduces a cooperative interaction between the sites, which shifts the affinity by an intrinsic linkage contribution K_x, considered to be a constant for every pair of sites. We assume that this linkage contribution K_x is the direct result of the conformational change induced on the TCR–CD3 by agonist peptides but not by non-agonists [24]. In these conditions, and according to the model developed in the supplementary material online, the ratios of high (h)- and low (l)- affinity bound ligands depend on the degree of multivalency (v), intrinsic affinity (K_A), intrinsic linkage contribution (K_x) and the number of MHC clusters (L) according to the following equation:

$$\log\left(\frac{[L^h_{Bound}]}{[L^l_{Bound}]}\right) \approx \log\left(\frac{[L^h]}{[L^l]}\right) + (v-1)\log\left(\frac{K^h_X}{K^l_X}\right) + v\log\left(\frac{K^h_A}{K^l_A}\right)$$

[Equation 1]

where L is the number of MHC clusters, and v is the total valence of the MHC cluster.

Equation 1 clearly indicates that multivalent binding greatly amplifies the differences in the binding of the peptides to their respective sites (Figure I). For example, in a tetravalent MHC cluster (Figure Ia), a tenfold difference in the intrinsic affinity of the agonist peptide ($K^h_A/K^l_A = 10$) translates into 1 000 000-fold increase in the occupancy of the TCR–CD3 cluster by ligand. This large amplifying effect could help to explain the exquisite selectivity of the MHC–TCR–CD3 interaction.

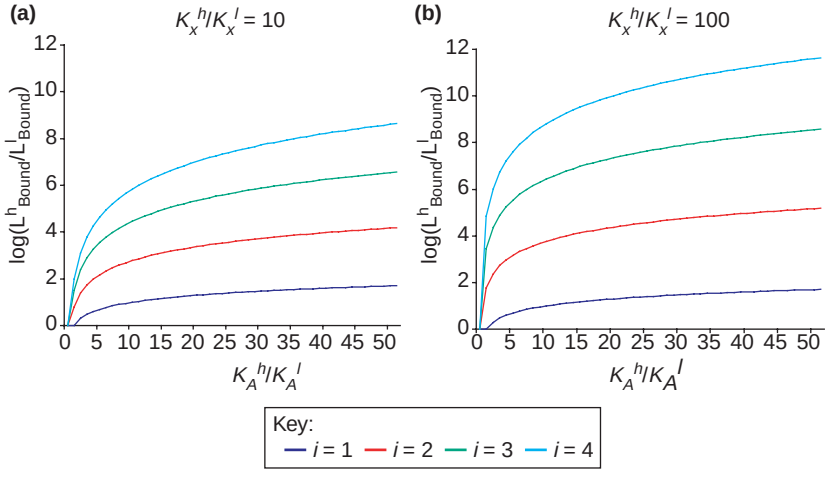

Figure I. Multivalency and the conformational change enables T cells to discriminate between agonist and self peptides. The expected ratio of bound high- and low-affinity ligands [$\log(L^h_{Bound}/L^l_{Bound})$] obtained according to Equation 1 is plotted against the intrinsic affinity ratio of an agonistic pMHC (K_A high, K^h_A) and a self peptide–MHC (K_A low, K^l_A). Two different linkage ratios (K^h_X/K^l_X) have been used in the simulations to illustrate the effect of the conformational change on ligand discrimination: K_X ratios between agonist and self peptide of **(a)** 10 and **(b)** 100 were used. Note that a K_X ratio of 10 is approximately equivalent to a free energy difference of 1 kcal/mol (the energetic cost of breaking or forming one hydrogen bond), whereas a ratio of 100 amounts to ~2 kcal/mol. The graphs clearly show that multivalent binding greatly amplifies the intrinsic binding differences of the peptides. For example, for a tetrameric MHC cluster (v=4) in which i=4 (i is the number of peptides bound in a MHC with valence v), a ratio of only tenfold in the intrinsic affinity of the peptides translates into 100 000-fold in the ratio of bound ligand, at equal ligand concentrations and with a linkage ratio of 10 (a). By contrast, for monovalent binding (i=1) the ratio of bound ligand would only be tenfold. When the linkage contribution K_X is 100 (b), a ratio of only fivefold in the intrinsic constants of the peptides translates into a 1000-fold difference in the ratio of bound ligand for i=2, into a 100 000-fold difference for i=3 and into a 10 000 000-fold difference for i=4. The large amplifying effect of multivalent engagement could help to explain the exquisite selectivity of the MHC–TCR–CD3 interaction.

Indeed, multivalent pMHC is necessary to activate T cells [35,36].

Binding of the MHC cluster begins with a monovalent interaction between one of the agonist-loaded MHC units and one of the TCR–CD3 units in the TCR–CD3 cluster. The initial binding event facilitates the binding of a second agonist-loaded MHC unit. This bivalent binding of two agonists to the TCR–CD3 cluster introduces a cooperative interaction between sites, resulting in a conformational change in the cluster. At any given time-point, weak pMHC ligands form a greater proportion of TCR–CD3–pMHC complexes in which the TCR–CD3 is bound monovalently rather than multivalently. Therefore, the requisite of bivalent binding for the induction of

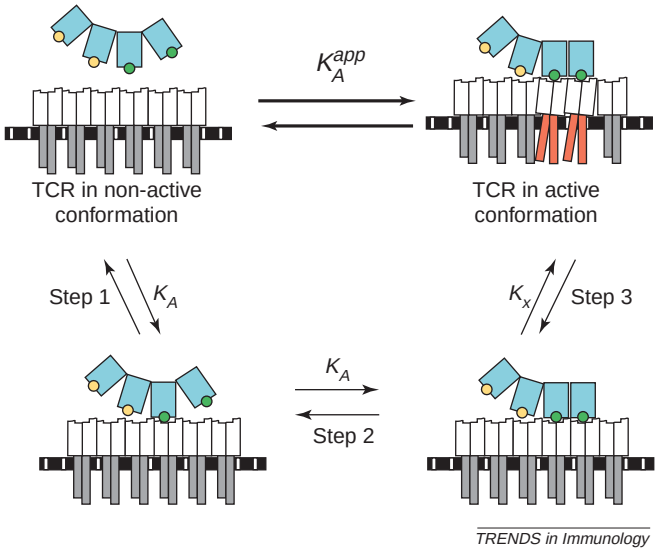

Figure 1. A model for ligand discrimination based on avidity and cooperativity. The model assumes that in addition to pre-existing TCR–CD3 clusters in the T cell, the APC also expresses MHC clusters. The activation-competent form of a MHC oligomer contains two or more MHC units, which are occupied with peptide antigens of sufficient affinity and reduced dissociation kinetics (agonists, green circles). After adhering to the APC through adhesion molecules, the T cell scans the APC surface until a TCR–CD3 cluster (white and gray) encounters an appropriate MHC oligomer (in our model, a MHC tetramer, blue). Binding of the MHC cluster begins with a monovalent interaction between one of the agonist-loaded MHC units and one of the TCR–CD3 units in the TCR–CD3 cluster with an intrinsic affinity constant K_A (step 1). This facilitates the binding of a second agonist-loaded MHC unit (step 2). The bivalent binding of two agonists to the TCR–CD3 cluster introduces a cooperative interaction between sites that results in a conformational change in the CD3 subunits (red), which shifts the TCR–CD3–pMHC affinity by the intrinsic linkage contribution K_X (step 3). The result of the cooperative effect is that the apparent binding affinity of the pMHC interaction with TCR–CD3 (K_A^{app}) will be increased with respect to the intrinsic affinity for the monovalent interaction (K_A) by a factor that exponentially reflects the valence of the interaction. Therefore, the cooperative effect resulting from the multivalency of receptor and ligand converts a small difference in the intrinsic interaction affinity into an extremely effective discriminatory mechanism. In this model, MHC units loaded with low-affinity self peptide–MHC (yellow circles) within the same MHC oligomer have no effect.

conformational change imposes a threshold of affinity. The result of the cooperative effect is that the apparent binding affinity of the interaction of the pMHC with the TCR–CD3 is increased by a factor that exponentially reflects the valence of the interaction. Thus, a small difference in the intrinsic affinity can be converted into an extremely effective discriminatory mechanism as a result of the cooperative effect resulting from the multivalency of receptor and ligand. Together, and through thermodynamic considerations alone, multivalency and the conformational change can explain the high specificity of antigen recognition (Box 1 and Figure 1).

According to our model, the triggering mechanism of the TCR–CD3 should distinguish between monovalent and multivalent engagement. We hypothesize that this distinction can be made through the conformational change in the TCR–CD3. This conformational change could be directly responsible for converting the multivalent engagement of the TCR–CD3 into productive TCR–CD3 signaling. In general, the available crystallographic data of monovalent TCRαβ–pMHC interactions preclude the existence of an obvious conformational change in the ectodomains of the TCRαβ heterodimer that could be transmitted to the CD3 subunits [23,37,38]. However, multivalent binding of pMHC to the ligand-binding TCRαβ subunits could give a 'push' to the CD3 subunits, producing a piston-like movement [39], a scissor-like movement [40] or a rotation that might alter the angle of interaction between the TCRαβ and CD3 subunits [25]. In turn, the conformational change might be transmitted from the CD3 ectodomains to the CD3 tails.

For such a mechanism to reflect the binding state of pMHC (multivalent versus monovalent), the conformational change must rapidly revert to the resting state when multivalent engagement reverts to monovalent (Figure 1). This is possible, and we have shown that the conformational change is reverted on ligand detachment (S. Minguet *et al.*, unpublished data)†.

Spreading of the triggering signal within the multivalent TCR–CD3

The reversibility of the conformational change makes serial triggering of TCR–CD3s by a single pMHC oligomer unviable [41] because the TCR–CD3 would not retain the memory of a previous engagement. Nevertheless, the multivalent nature of TCR–CD3 engagement does provide other opportunities for signal amplification. This amplification could occur if the engaged TCR–CD3 units could transmit the conformational change to unengaged TCR–CD3 units in the same cluster (Figure 2).

An obvious mechanism for signal spreading results from the increased local concentration of CD3 tails and their corresponding ITAMs in the multivalent complexes. The engaged TCR–CD3 units recruit the tyrosine kinase Lck, which might phosphorylate not only the ITAMs of the engaged TCR–CD3s, but also those of the nearby unengaged receptors within the cluster [42]. However,

† Data along these lines were presented at the 'Genetic control of T-cell activation' workshop in Henningsvaer, Lofoten Islands, Norway, on 19 August 2005: S. Minguet, *et al.* 'The ligand induced conformational change of the TCR is induced by multivalent receptor engagement and is reversible'. The results have also been presented at the 5th Freiburg Immunology meeting in Emmendingen, Germany, on 11 October 2005: S. Minguet and W. Schamel 'MHC peptide-binding induces a reversible conformational change in the TCR'.

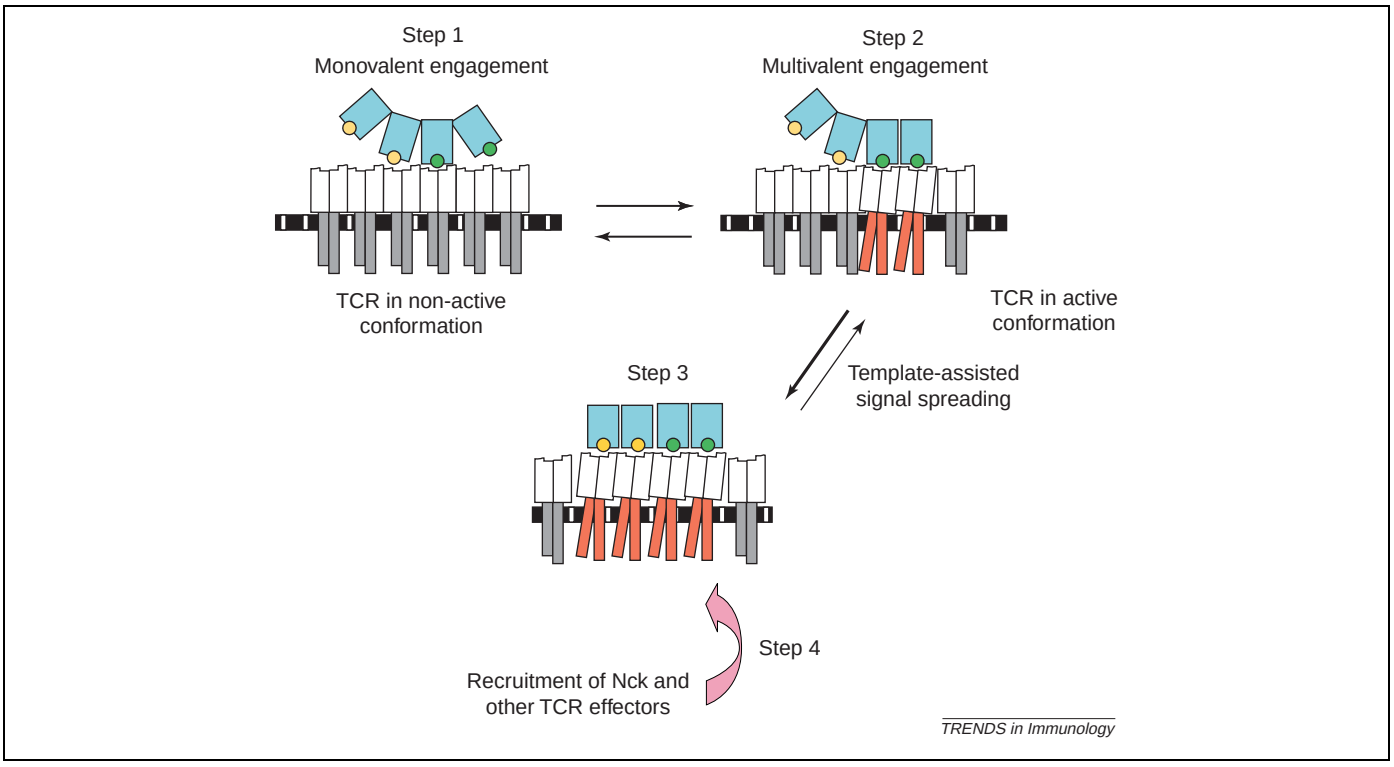

Figure 2. A conformation-based signal-spreading mechanism for signal amplification and the recruitment of low-affinity endogenous ligands. Binding of the MHC cluster (blue) begins with a monovalent interaction between one of the MHC units loaded with agonist (green circle) and one of the TCR–CD3 units (white and gray) in the TCR–CD3 cluster (step 1). This initial binding event facilitates the binding of a second agonistic MHC unit, and the bivalent binding of two agonists to the TCR–CD3 cluster promotes a conformational change in the CD3 subunits (red, step 2). If the MHC oligomer is loaded with only one agonist peptide or all peptides are of low affinity, the multivalent engagement of the TCR–CD3 rapidly reverts to a monovalent state or the interaction is lost. In either case, the TCR–CD3 does not undergo a conformational change (step 1). These steps are common to the model present in Figure 1. However, in the signal spreading model, the successful induction of the conformational change in two or more TCR–CD3 units within the TCR–CD3 cluster can catalyze the conformational change in neighboring non-engaged TCR–CD3 units. This spreading of the conformational change to unengaged TCR–CD3 units facilitates the binding of low-affinity self peptides (yellow circles), which do not need to cross the free energy barrier imposed by the conformational change (step 3). The binding of self peptide-loaded MHC units contributes to the stabilization of the active conformation of the whole TCR–CD3 cluster. The exposure of the CD3 tails resulting from the upstream conformational change results in the recruitment of the adaptor protein Nck to the polyproline sequence of CD3ε [25], and possibly other TCR–CD3 effectors that contribute to transmit a distinct signal (step 4). According to this model, the conformational change in the TCR–CD3 would directly translate kinetic and avidity parameters into an effective TCR–CD3 signal.

the TCR–CD3 clusters are organized in linear arrays [3], rather than in random assemblies. This linear arrangement suggests that the multivalent TCR–CD3 can facilitate the amplification of the activation signal by transmitting the active conformation of the engaged TCR–CD3 units to the unengaged units along the oligomerization axis of the cluster. In other words, two TCR–CD3 units in the active conformation could transmit the conformational change to non-engaged TCR–CD3 units by what could be named 'template-assisted signal spreading'.

This mechanism would be reminiscent of the 'template-assisted conversion model' proposed for the transmission of prion diseases [43]. In this model, the cellular precursor PrP^{c} is thermodynamically less stable than the infectious protein PrP^{sc}, which catalyzes the conversion of PrP^{c} into PrP^{sc} by reducing the free energy transition barrier. Likewise, if the conformational change in two TCR–CD3 units promoted by multivalent binding of agonist peptides were sensed by the rest of the TCR–CD3 units within the same cluster, the occupancy of the remaining TCR–CD3 units by low-affinity self peptide–MHC ligands would be facilitated.

Unlike the prion example, however, we are not suggesting that the TCR–CD3 is expressed in a misfolded state; rather, we believe that the signal spreading mechanism would be more similar to traditional allosteric systems. Under these conditions, binding of the low-affinity ligands to non-occupied TCR–CD3 sites in the same cluster would be encouraged because the original energetic barrier required for the conformational change would have been satisfied. This could constitute a positive feedback mechanism because the binding of self peptide–MHC ligands to a TCR–CD3 unit in the open conformation might help to stabilize this conformation (Figure 2). In this way, the initial binding of a MHC oligomer containing two agonist peptides could facilitate the binding of the lower affinity pMHC within the same MHC oligomer. Such a mechanism could explain the cooperation observed between low-affinity self peptide ligands and full agonists during T-cell activation [44].

The combination of a conformation-based proofreading mechanism with a template-assisted signal spreading mechanism would constitute a two-step process. This process could guarantee the specificity of the T-cell response to agonist pMHC through thermodynamic restrictions imposed by the multivalent engagement of agonist pMHC and the conformational change. Additionally, sensitivity would be favored by the spreading of the signal to nearby TCR–CD3 units within the same cluster.

Our model provides a role for non-agonist peptides in the amplification and stability of the activation signal. However, this model differs from the 'pseudodimer model' proposed by Davis and colleagues [9,44,45] in that the TCR–CD3 pre-exists in clusters before stimulation. Furthermore, clustering does not depend on the interaction of the coreceptors with nearby pMHC complexes. Some artificial soluble pMHC dimers that simultaneously present one agonist and one self peptide could stimulate T cells, whereas others that used a different self peptide could not [44]. Therefore, the pseudodimer model proposes that only one agonist pMHC is required for activation. According to our model, certain high-affinity self peptides could induce the conformational change in combination with an agonist if a given receptor affinity threshold can be overcome.

The requirement for more than one pMHC agonist to elicit full TCR signaling raises another intriguing question: if T cells respond to a few tens of pMHC agonists, how probable is it that two or more pMHC agonists within a sea of hundreds of thousands of self peptide–MHC lie in the same MHC cluster? Even considering a random distribution of the agonist peptides, it is not improbable for a T cell to meet an antigen presenting cell (APC) expressing one MHC cluster containing two or more agonist peptides, as a ballpark calculation easily shows (supplementary material online). As an example, for APCs displaying 100 000 self peptides and only 100 agonist peptides, a T cell would need, on average, to scan the surface of approximately five APCs to become activated. Thus, a simple random model is not unreasonable and matches well with experimental observations [46–49]. Other authors have indicated, however, that T cells can become activated by ten pMHC agonists or even less [9,10,50,51]. Nevertheless, the accuracy of their calculations might have been hampered, either because they refer to average values of pMHC agonist molecules being presented by an APC population [50,51], or because the method used for direct detection underestimates the number of agonist peptides within a MHC cluster [9,10].

To explain responses to concentrations of agonist peptides that are <100, additional mechanisms can be invoked. A mechanism that might ensure this could be asymmetric MHC loading. Most antigen sources are particulate material that is endocytosed by professional APCs and transported to the MHC class I and II loading compartments (the endoplasmic reticulum and the class II MHC endocytotic compartment). Therefore, the particulate nature of the antigen makes it improbable that only one antigen molecule will be presented by an APC, and it is doubtful whether the access of antigen to the loading compartments is homogenous. A second mechanism might be one that concentrates a selected set of peptide antigens in specific membrane microdomains, such as those rich in tetraspanins [33,34]. Indeed, tetraspan microdomains have been shown to promote the cosegregation of antigen peptides with self peptides such as CLIP, which in turn helps to modulate the T-cell response [52].

Concluding remarks

We have proposed a new model here that tries to explain the salient features of the T-cell response to antigens. The model depends on simple thermodynamic arguments that take into consideration receptor and ligand multivalency, and a conformational change in the TCR–CD3 as a readout. In this model, the free energy barrier imposed by the conformational change establishes a threshold responsible for the discrimination of agonist pMHC from self peptides–MHC. Although, for simplicity, we have not considered kinetic parameters in the model, the conformational change in the TCR–CD3 could indeed be a clock that also measures the time of bivalent or multivalent TCR–CD3 engagement, conveying this where appropriate into a full activation response. A combination of a high-avidity interaction with the conformational change is therefore responsible for ligand discrimination.

In addition, a hypothetical mechanism of signal spreading permits the TCR–CD3 complexes within the same cluster that are engaged by self peptide–MHC to participate in signaling, explaining the high sensitivity of T cells. This signal spreading might serve as a mechanism to regulate T-cell sensitivity to antigen. Therefore, we propose an avidity-maturation mechanism that increases the sensitivity of activated and memory T cells to antigen by increasing the average size of their TCR–CD3s. The nature of the molecular determinants that control TCR–CD3 clustering and regulate the transmission of the conformational change across the membrane remains a matter of speculation and is currently the subject of intense investigation.

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Supplementary data

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