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T cell receptor triggering by force

Zhengyu Ma and Terri H. Finkel

Department of Pediatrics, The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

Antigen recognition through the interaction between the T cell receptor (TCR) and peptide presented by major histocompatibility complex (pMHC) is the first step in T cell-mediated immune responses. How this interaction triggers TCR signaling that leads to T cell activation is still unclear. Taking into account the mechanical stress exerted on the pMHC-TCR interaction at the dynamic interface between T cells and antigen presenting cells (APCs), we propose the so-called *receptor deformation model* of TCR triggering. In this model, TCR conformational change induced by mechanical forces initiates TCR signaling. The receptor deformation model, for the first time, explains all three aspects of the TCR triggering puzzle: mechanism, specificity, and sensitivity.

Introduction

T cells use T cell receptors (TCRs) to probe short peptides presented by major histocompatibility complexes (pMHCs) on the surface of antigen presenting cells (APCs). A 'specific' pMHC-TCR binding leads to an intracellular signaling cascade that ultimately activates T cells. pMHC-TCR interaction is therefore the molecular basis for immune recognition. How this interaction triggers TCR signaling, however, has eluded researchers for the more than two decades since the TCR was cloned [1]. Classical triggering models, such as receptor crosslinking and conformational change, applicable for hormone receptors, are problematic in explaining TCR triggering. Although TCR crosslinking by antibodies or pMHC tetramers initiates TCR signaling [2], this is not likely to occur at the T cell-APC interface. It is generally accepted that MHC molecules exist as monomers and the majority of peptides presented are irrelevant endogenous peptides. The chance of two specific peptides being presented by two MHC molecules in very close proximity (~10 nm) [3] for a sufficient period of time to act as a dimer is therefore very small, especially considering that the TCR can be triggered by very few specific pMHCs (1 to 20) on an APC [4-7]. Therefore, although it cannot be totally excluded that TCR crosslinking might be a working mechanism when a very large quantity of a single peptide species is presented by the APC (e.g. following peptide pulsing), TCR crosslinking is unlikely to be the mechanism that operates under most physiological conditions. Problems also arise with the classical TCR conformational change model as it would predict that soluble monovalent pMHCs trigger TCR. Most studies, however, have shown that soluble monovalent pMHCs bind to TCRs but fail to initiate TCR signaling [2, 8-10]. A combination of the two models, the *permissive geometry* model, argues that crosslinking induces conformational change [11]. Like the crosslinking model, however, it suffers from the unlikely presence of multimeric specific pMHCs on the surface of APC.

Corresponding author: Ma, Z. (maz@email.chop.edu).

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The difficulty in understanding TCR triggering lies in the complexity of the TCR-CD3 complex and in the physical environment where the pMHC-TCR interaction takes place. The TCR itself has no known intracellular signaling domains and instead relies on immunoreceptor tyrosine-based activation motifs (ITAMs) in six non-covalently associated CD3 chains for signaling. This compound transmembrane configuration hinders direct structural analysis using currently available technologies. The pMHC-TCR interaction occurs at the interface between T cell and APC where the movement of pMHC and TCR is restricted to the two dimensional plasma membranes that anchor them. Binding kinetics in this setting cannot be directly determined from three dimensional studies using techniques such as surface plasmon resonance (SPR) [12].

Paradoxically, we believe that another element of this complexity might lead to an answer to the TCR triggering puzzle. T cells are highly mobile, as demanded by their function in patrolling the body and as demonstrated by *in vivo* imaging studies [13,14]. The T cell-APC interaction is also highly dynamic, consisting of repeated contact and separation, T cell migration on APCs, changes in T cell shape, and T cell membrane ruffling [15]. Membrane dissociation coupled with these events should exert significant mechanical stress on the pMHC-TCR interaction [1,16]. Taking this into consideration, we propose the *receptor deformation model* of TCR triggering [17] (Figure 1). In this model, mechanical forces induce conformational changes in the TCR-CD3 complex that favor downstream signaling events. The particular kinetic parameters of the pMHC-TCR interaction under force explain the other two aspects of the TCR triggering puzzle: specificity and sensitivity.

The receptor deformation model of TCR triggering

An interesting aspect of the TCR triggering puzzle is the sharp contrast between the inability of soluble monomeric pMHCs to trigger TCR [2,8-10] and the ability of very few of the same ligands on APCs to activate T cells [4-7]. Using pMHCs anchored on artificial surfaces, including fluid lipid bilayers and fixed plastic plates, our previous study isolated three elements contributing to the potency of pMHCs on APCs: surface anchorage, adhesion, and intact actin cytoskeleton function [6]. These observations, together with two-photon microscopy data showing highly dynamic T cell-APC interactions *in vivo*, led us to propose the receptor deformation model, in which mechanical forces drive TCR triggering (Figure 1). In this model, T cell motility facilitates the encounter and binding of a specific pMHC by a TCR. The binding *per se*, however, does not trigger TCR. A signal is initiated when the TCR-CD3 complex is deformed by a pulling force associated with cell motility. The deformed TCR assumes a conformation that favors downstream phosphorylation events, presumably by exposing ITAMs and other domains. We propose that the initial pulling force originates from myosin activity inherently associated with the motility of the T cell, especially the lifting of the cell body at the rear during its locomotion in the form of an amoeboid walk [18]. This initial TCR triggering driven by motility-associated forces delivers a series of signals to the cytoskeleton to facilitate more TCR triggering events. First, the fast and directional migration is arrested so that the same antigen-bearing APC can be further probed. Second, lamellapodia are formed to expand the T cell-APC contact area. Finally, membrane ruffling at the periphery of the contact area generates force for TCR triggering [19]. The deformation of TCR-CD3 is most likely caused by a pulling force applied perpendicular to the cell membrane, as 95% of TCRs diffuse freely on the T cell surface [20] and therefore are not horizontally anchored. In the following, we discuss how the receptor deformation model explains the three aspects of the TCR triggering puzzle: mechanism, specificity, and sensitivity (see Box).

Mechanism: receptor deformation by force

The intracellular domains of the TCR-CD3 complex do not possess intrinsic enzymatic activities but have ten immunoreceptor tyrosine-based activation motifs (ITAMs) in total that can be phosphorylated by the Src family tyrosine kinase Lck anchored on the plasma membrane. Docking of zeta-associated protein 70 (ZAP70) on phosphorylated ITAMs leads to a cascade of downstream signaling events [21]. One school of thought, incorporated into the TCR crosslinking, coreceptor heterodimerization, and kinetic segregation models [22], holds the view that ITAM phosphorylation induced by ligand binding is achieved by increased Lck interaction with freely accessible ITAMs. These models, however, are inconsistent, respectively, with the monovalent nature of pMHC, the dispensable role of coreceptors [23-25], and the fast kinetics of pMHC-TCR interaction under force (discussed below). Moreover, these models do not account for all three aspects of the TCR triggering puzzle (see ref. [17] for detailed discussion of these models). Here, we argue that the intracellular signaling components of the TCR-CD3 complex normally assume a ‘closed’ conformation and conformational changes induced by force are necessary to expose the concealed ITAMs.

The case for conformational change

The most compelling evidence for concealed ITAMs in unstimulated T cells comes from a recent report that looked into the interaction between CD3 ϵ and lipids [26]. Mutation and imaging experiments demonstrated that the CD3 ϵ cytoplasmic domain binds to the plasma membrane through charge interactions. Circular dichroism and NMR spectroscopy revealed that lipid binding induces partial folding of CD3 ϵ . Importantly, two key tyrosine residues insert deeply into the hydrophobic core of the membrane lipid bilayer and cannot be phosphorylated by Lck. Earlier studies from another group indicated that the CD3 ζ chain also binds to lipid vesicles and the binding partially inhibits ITAM phosphorylation, although the binding may not induce protein folding [27,28]. These studies are in line with experimental evidence showing that TCR engagement induces the exposure of a proline-rich stretch of the CD3 ϵ [29,30]. Therefore, although prior studies investigating individual CD3 subunits in isolation found their cytosolic portion unstructured and ITAMs exposed [31,32], the intact TCR-CD3 complex on the plasma membrane is likely to have a more organized structure through interactions among the complex's subunits and with other cellular components

If ITAMs are concealed and inaccessible to Lck in unstimulated T cells, increasing Lck activity should not lead to autonomous TCR signaling without TCR engagement. This is indeed supported by data in one of the first papers studying the function of Lck, showing that overexpressing constitutively active Lck does not lead to autonomous TCR signaling [33]. In addition, autonomous TCR signaling was not observed in immature or mature T cells from CD4 and CD8 double knockout mice [25]. In these mice, because of the lack of Lck sequestration by coreceptors, T cells have markedly greater amounts of “free” Lck and much increased TCR signaling upon TCR engagement. For these observations to support a closed conformation of the TCR-CD3 complex in unstimulated T cells, the TCR-CD3 complex and “free” Lck must not be separated by a physical barrier. In fact, there are no data supporting the existence of such a barrier. It is more likely that TCR and Lck encounter each other frequently on the plasma membrane, since both TCR [20] and Lck [34] are highly mobile at the single molecule level on unstimulated T cells, where both partially partition into lipid rafts [35]. Taking into account the data described above, this suggests that TCR-CD3 conformational change is the most likely explanation for receptor triggering.

Conformational change by force

pMHC binding *per se* does not seem to induce consistent large scale TCR conformational changes. In most crystallography studies, compared with free TCRs, TCRs bound by soluble

pMHCs show only minor conformational changes restricted to the variable regions [36]. A recent study however reported that pMHC binding could induce a subtle change in a loop region of the constant domain of two TCRs [37]. Such a change, however, was not evident in the structures of about a dozen other TCRs engaged by pMHCs. It is not obvious how these variable and subtle changes in the TCR could be transmitted to the intracellular domains of CD3 to induce a consistent signaling conformation. Moreover, since soluble monomeric specific pMHCs do not trigger TCR signaling [2,8-10], the functional relevance of these subtle changes is questionable.

Mechanical forces provide a straightforward mechanism for pMHC-TCR binding induced CD3 conformational change. A pulling force on the TCR might directly deform its constant region and the conformational change so generated could be transmitted through TCR-CD3 interactions in the plasma membrane, or between TCR constant regions and the extracellular domains of CD3 subunits [38,39]. If the TCR structure is rigid, the force could be transmitted down to the cell membrane and change the relative positioning or configuration of CD3 subunits (Figure 1). Alternatively, a force might disrupt the packing order of lipid molecules immediately surrounding the TCR-CD3 complex and release the CD3 ϵ ITAM from lipid association, opening it up for phosphorylation. These conformational changes may be transient and reversible, in which case a TCR could be triggered multiple times. More likely, however, the conformation of the TCR-CD3 is permanently altered and the complex will be transported to the center of the immunological synapse for internalization and recycling, as suggested by previous studies [19,40,41]. In addition, it is also possible that interaction with the TCR results in deformation of the MHC molecule allowing it to take on a conformation that binds to coreceptors with higher affinity (Figure 1).

Recent research in the field of mechanotransduction has accrued a large body of evidence for force-induced protein conformational change [42]. By translating mechanical forces and deformations into biochemical signals, mechanotransduction allows cells to sense their physical environment and to modulate cellular functions as diverse as adhesion, migration, proliferation, and differentiation. It is crucial in organ development [43], vascular physiology [44] and mechanosensing by neurons [45]. Of particular interest is the force-induced opening of a cryptic region on the focal adhesion protein, p130Cas, to phosphorylation by Src family kinases [46]. Beyond mechanotransduction, molecular dynamics simulations suggest that mechanical force can convert the integrin β subunit to a conformation with high affinity for extracellular matrix binding [47]. In addition, there is evidence suggesting that Notch signaling is triggered by mechanical force [48-51]. Endocytosis of the Notch ligand, Delta, might exert a pulling force on Notch to expose a protease cleavage site by either deforming the extracellular domain or by directly pulling away a subunit that covers the cleavage site. Since both TCR and MHC are constitutively internalized for recycling [52-54], it is possible that endocytosis could provide force to deform the TCR. However, at least in the early stages of T cell-APC contact, this is likely to be overshadowed by forces originating from T cell motility, which are probably much larger.

Although the amount of force required to generate the aforementioned TCR-CD3 structural changes is unknown, there are reasons to believe that T cells are capable of generating forces of such a magnitude. Some protein domains, such as the α -helix domains in spectrin, can be unfolded by forces in the range of 30 pN (pico Newton), which can be generated by about 10 myosin molecules [55]. A striking testimony to the physical prowess of T cells is their transcellular migration through endothelial cells by mechanically boring pores into the endothelial cell body with "invasive podosomes" [56]. Since endothelial cells are capable of presenting antigens [57], pMHC-TCR interactions at the contact interface between T cell podosome and endothelial membrane must be under considerable mechanical stress, although the vector of force may vary depending on where the interaction takes place on the surface of

the irregularly shaped podosome. A more relevant question, however, is how much of the total force can be transferred to the TCR through the pMHC-TCR interaction before this relatively weak binding ruptures. Data on the mechanical strength of pMHC-TCR interaction is unavailable. It has been well documented, however, that T cells can acquire MHC molecules from APCs [58,59]. This would suggest that pMHC binding to TCR can sustain a significant force, if the transfer of MHC molecules is due to force-induced uprooting of MHCs, as proposed [60].

Specificity: rupture force as the determining factor

APCs present a huge variety of endogenous and exogenous peptides on the cell surface. T cells are therefore faced with the task of distinguishing structurally very similar pMHC ligands. What is the binding parameter that defines a specific pMHC-TCR binding that can lead to TCR triggering and T cell activation? According to the receptor deformation model, the critical factor is whether the particular pMHC-TCR binding has sufficient mechanical strength to deliver an external force to the TCR and induce a conformational change. In other words, the parameter that defines specificity is binding strength under force, or 'rupture force' (the amount of force it takes to rupture the ligand-receptor binding). To trigger a TCR, the pMHC-TCR binding must have a rupture force larger than a signal-initiating threshold, and the larger the rupture force, the stronger the signal. A "partial agonist", for example, would bind TCR with an intermediate rupture force.

As a parameter describing ligand-receptor interaction under force, 'rupture force' is superior to other kinetic parameters in describing pMHC-TCR interaction at the T cell-APC interface. The spontaneous on-rate and off-rate of pMHC-TCR interactions have been extensively characterized, mostly using SPR which monitors the binding of TCR or pMHC in solution to its binding partner immobilized on a surface. This setting mimics three-dimensional interactions, such as the binding of soluble ligands, e.g., hormones, to their receptors. Since the binding half-life $t_{1/2} = \ln(2)/\text{off-rate}$, the value of measured off-rate reflects the intrinsic stability of pMHC-TCR binding under zero force (aside from thermal agitation). The real stability of pMHC-TCR interaction at the T cell-APC interface, however, is not likely to be determined by the zero force off-rate as measured by SPR. Instead, it must be largely determined by the distance between the two membranes and the force behind them. For instance, when the two membranes are stably aligned at a distance equal to the combined dimensions of pMHC and TCR, the pMHC-TCR binding should be very stable, regardless of the zero force off-rate. The interaction either cannot dissociate because it is 'pushed' together by the membranes, or, even if dissociation occurs, the same ligand-receptor pair will quickly rebound, due to the very fast on-rate resulting from the very close distance between the ligand and receptor and the near perfect alignment of their binding interfaces. On the other hand, if the two membranes are detaching from each other and a mechanical stress is applied on the pMHC-TCR interaction (as happens during dynamic T cell-APC interaction), the stability of the pMHC-TCR interaction will be dramatically reduced, since the real off-rate increases exponentially with the amount of pulling force exerted on the binding pair [61].

Among the kinetic parameters measured by SPR, including affinity, on-rate, and off-rate, only off-rate was found to partially correlate with pMHC triggering capacity in an inverse fashion, i.e., "low off-rate $\rightarrow$ high triggering capacity" [1,62-64]. As discussed above, since zero-force off-rate does not describe the stability of pMHC-TCR binding at the T cell-APC interface, this correlation cannot be interpreted as showing that TCR specificity is determined by the stability of pMHC-TCR binding. On the other hand, this correlation is actually consistent with our hypothesis that "high rupture force $\rightarrow$ high triggering capacity", if there is an inverse correlation between off-rate and rupture force: "low off-rate $\rightarrow$ high rupture force". Indeed, such a correlation has been demonstrated in a study where the rupture forces of antibody-

antigen interactions with different off-rates were measured using atomic force microscopy (AFM) [65]. Therefore, our hypothesis that rupture force determines TCR specificity is supported by existing experimental data.

Sensitivity: fast and efficient TCR serial triggering

Multiple studies have shown that T cells are capable of detecting a very low number (1-20) of specific pMHCs on APCs [4-7]. To explain this high sensitivity, the TCR serial triggering model has been proposed, in which signals from multiple TCRs sequentially triggered by a single specific pMHC are integrated in order to reach a certain threshold [66]. The average zero force half-life of pMHC-TCR binding is 10 seconds and can be as long as 50 seconds [67]. Therefore, if a specific pMHC dissociates from TCR spontaneously, not many TCRs can be triggered in the few seconds to minutes of time required for signaling events such as calcium flux and ERK activation [4,68,69]. In addition, signal integration may be poor if signals from individual TCRs are short-lived and the interval between each TCR triggering is long. On the other hand, if a disengaging force is applied to the pMHC-TCR interaction, a much faster dissociation should ensue, since the off-rate increases exponentially with force [61]. As a result, a much more rapid and efficient TCR serial triggering process can be achieved.

Conclusion

We propose that TCR triggering is intimately coupled with the motor system of T cells, which generates mechanical forces not only to help T cells find specific pMHCs through cell motility, but also to initiate TCR signaling through receptor deformation. The receptor deformation model of TCR triggering is consistent with the indispensable role of actin and myosin in TCR signaling [6,69-71]. Although currently there is no direct experimental evidence supporting it, the receptor deformation model can be tested by taking advantage of exciting recent advances in computer simulation, AFM, and structural analysis using NMR and fluorescence-based techniques. For example, using AFM-based force spectroscopy, the rupture force of pMHC-TCR binding can be measured to determine whether it correlates with the potency of pMHC in T cell activation. Force can also be applied directly to the TCR using an AFM tip functionalized with pMHCs, while concurrently monitoring T cell responses in the form of calcium mobilization. Incorporation of mechanical force into the mechanism of TCR triggering resolves a number of previous intractable issues, and for the first time, explains all three aspects of the puzzle: mechanism, specificity and sensitivity.

Box. The receptor deformation model: theoretical strengths and experimental evidence

The receptor deformation model is theoretically appealing and is supported by current biochemical and biophysical evidence.

Theoretical strengths

Operates via an important physiological factor that has been previously omitted namely the mechanical stresses sustained by the pMHC-TCR interaction at the dynamic T cell-APC interface.

Explains all three aspects of the TCR triggering puzzle

- i. Mechanism: mechanical force-induced TCR-CD3 conformational changes
- ii. Specificity: determined by the mechanical strength of pMHC-TCR binding (rupture force)

- iii. Sensitivity: achieved by fast and efficient TCR serial triggering by a few specific pMHCs

Experimental evidence in line with the model

- i. The dynamic T cell-APC interaction *in vivo* [13,14]
- ii. The ITAM domain of CD3 ϵ is concealed in the plasma membrane in a manner that would seem to require drastic conformational change to allow phosphorylation by Lck [26].
- iii. TCR engagement induces the exposure of a proline-rich stretch of CD3 ϵ [29,30].
- iv. pMHCs with elongated extracellular stems have reduced TCR triggering capacity [72]. Elongated pMHCs would require the T cell membrane to travel a longer distance to apply force on pMHC-TCR binding.
- v. The potency of pMHC to trigger TCR partially correlates with the off-rate of pMHC-TCR binding [1,62–64] and off-rate correlates with rupture force [65].

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Figure 1.

The receptor deformation model of T cell receptor (TCR) triggering. (i) Adhesion molecules (CD2, CD48, ICAM-1, and LFA-1) align the plasma membranes of T cell and antigen presenting cell (APC) for pMHC and TCR to interact. However, this interaction *per se* does not trigger TCR signaling. (ii) A pulling force associated with T cell motility exerts mechanical stress on pMHC-TCR binding. The weak binding between TCR 1 and nonspecific pMHC is ruptured without inducing a TCR conformational change or TCR signaling. The binding between TCR 2 and cognate pMHC is strong enough to deliver sufficient force to deform TCR and induce TCR signaling. (iii) T cell movement changes the relative positioning of T cell and APC. The same specific pMHC now binds and deforms TCR 3. The integration of signals from TCR 3 and TCR 2 contributes to the high sensitivity of TCR triggering to very low numbers of specific pMHCs on APCs.