Published in final edited form as:

Trends Immunol. 2014 December ; 35(12): 597–603. doi:10.1016/j.it.2014.10.007.

Force and affinity in ligand discrimination by the TCR

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Abstract

T cell recognition of antigen is a physical process that requires formation of a cell-cell junction that is rich in active force generation. Recently, a biomolecular force probe was used to examine how the TCR-pMHC interaction responds to force and the consequences of force dependent interactions for T cell activation. While adhesion and co-stimulatory molecules in the immunological synapse impact the overall force of the interaction, these results suggest that the TCR uses a force-dependent bond – a catch bond – and that it may therefore be important to consider the TCR-pMHC interaction in isolation in the early phases of the decision process. Here we discuss these findings in the context of other work on the impact of forces on the TCR and quantification of interaction in interfaces.

Introduction

T lymphocytes must recognize their activating ligands on the surface of other cells (Xie et al., 2012). This means that the T cell and antigen presenting cells (APC) must “get physical” with each other and build an immunological synapse involving generation of forces in the piconewton (pN) range (Bashour et al., 2014). In a recent study, Zhu and colleagues examined the effect of force on a single T cell receptor (TCR) interaction with peptide-MHC (pMHC) ligand using a biomembrane force probe (BFP), a device invented to observe molecular tethers (Liu et al., 2014). They provide support for a model wherein the T cell receptor uses a type of bond in which force dependent allostery increases the duration of the intermolecular interaction: a catch bond (Dembo et al., 1988, Thomas et al., 2006) (Box 1 and Box 2). The authors then use this device to test requirements for signaling using cytoplasmic Ca^{2+} elevation as the readout. The study thus has important implications for the physical nature of self, non-self discrimination and signal transduction, but also raises questions about how forces are distributed in the immunological synapse and how much force must be withstood by individual receptor-ligand interactions. These questions are fundamental to understanding and manipulating antigen specific recognition by T cells.

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Since the mid 1990's, molecular immunology has been trying to understand how T cells distinguish self-pMHC from agonist foreign-pMHC at a physical level (Matsui et al., 1994). The phenomenon of antagonism, in which certain pMHC actually reduce the sensitivity of T cells, is also controlled by characteristics of the TCR-pMHC interaction that are not entirely understood (Kersh et al., 1998). The related question facing those interested in T cell development is how does a thymocyte distinguish self-ligands that induce positive selection (survival) or negative selection (death) to create a largely self-tolerant T cell repertoire (Daniels et al., 2006). The early enabling technology in this effort was surface plasmon resonance, which allowed the interactions of soluble TCR and pMHC extracellular domains to be analyzed despite their relatively low affinity and fast kinetics (Alam et al., 1999, Garcia et al., 1997, Matsui et al., 1994). Understanding the discrimination mechanism is potentially important for vaccine design, pathogen escape from the immune responses and treatment of autoimmunity. Measurements of TCR interaction with agonist pMHC in solution produce correlations between functional readouts with affinity or off rates, but no parameter consistently predicts T cell responses across different TCR (Govern et al., 2010, Qi et al., 2006). Models that take into account the on and off rates with diffusion to calculate a dwell time that incorporates rebinding correct some errant points, but still require support by direct measurements (Aleksic et al., 2010). The work from Liu et al raises an important question: how does force affect these interactions? In order to address this question we need to consider how interactions in interfaces are quantified. The dominant concept since the 1970s is based on two-dimensional (2D) affinity, referring to the use of 2D units to describe the density of molecules on a membrane surface (Bell, 1978). While the solution or 3D dissociation constant can be related directly to binding energy of a reaction between two proteins, the 2D affinity is dependent upon details of the contact interface and as such different measurement contexts provide different values- although all in 2D units (Dustin et al., 2001). The rationale for attacking such a moving target is that this is the way the TCR functions and the different types of measurements relate to different challenges inherent to a system based on direct cell-cell recognition at close range (Shaw and Dustin, 1997).

The reliance of T cells on formation of an immunological synapse to communicate via direct interaction of TCR and pMHC means that this interaction evolved to cope with physical forces. Liu et al explore the more specific hypothesis that this co-evolution with force would lead the immune system to use the response of the TCR-pMHC interaction to force for ligand discrimination. Early observations that T cells only respond to pMHC on solid supports above a threshold size ($>3\text{ }\mu\text{m}$) (Mescher, 1992) may be related to the length of myosin II thick filaments that can only generate force above a threshold length (Galbraith et al., 2002), and are important in immunological synapse formation (Ilani et al., 2009). TCR triggering is dependent upon an intact actin cytoskeleton and actin based lamellipodia that are force sensing structures are the most sensitive part of the T cell (Negulescu et al., 1996). TCR triggering by application of force was directly demonstrated by Reinherz and colleagues using laser tweezers to pull on non-stimulatory anti-CD3 coated beads tangentially to the cell surface to induce Ca^{2+} signaling (Kim et al., 2009). Roffler and colleagues came to similar conclusions using hydrodynamic flow in combination with non-stimulatory extended pMHC to activate T cells (Li et al., 2010). T cells have also been shown to be able to respond differentially to anti-CD3 coated substrates of varying stiffness

(Judokusumo et al., 2012, O'Connor et al., 2012). These results implicated force sensing by T cells, but applied the force to multiple TCR and many interpretations were possible including intrinsic force sensing by the TCR or force sensing by supramolecular assemblies in the T cell. In this context, the work by Liu et al, focusing on single TCR-pMHC bonds, moves towards a demonstration of intrinsic force sensing by the TCR.

To put these recent findings in context, its important to consider the ways in which 2D interactions have been approached in the past and how this new information can be integrated.

Measuring 2D interactions with ligands within a supported lipid bilayer

The interface of a T cell with a supported lipid bilayer (SLB) containing purified, laterally mobile biological ligands has been tractable because there is a very good physical model for the SLB and the SLB can activate T cells (Grakoui et al., 1999). Purified proteins attached to the upper-lipid leaflet of a fluid SLB are highly mobile with diffusion coefficients on the order of $1 \mu\text{m}^2/\text{s}$, the force required to move molecules laterally of $\sim 5 \text{ fN}$ (Jonsson et al., 2012) and a vertical force of $\sim 10 \text{ pN}$ needed to extract a lipid from the SLB. When these highly mobile ligands are fluorescently labeled they accumulate in adhesive interfaces in proportion to interactions with cell surface receptors. The 2D dissociation constant (2D Kd) can be estimated from the ligand accumulation as a function of ligand density in the SLB. Members of the Ig-superfamily like CD2 and CD28 have been most amenable to this approach and yield values of $0.6 \text{ molecules}/\mu\text{m}^2$ (Dustin et al., 1996, Dustin et al., 2007). The use of fluorescence photobleaching after recovery in these contact areas measured a kinetic off-rate of 0.074 s^{-1} (Tolentino et al., 2008), which is 100-fold slower than the solution off-rate of 6 s^{-1} (van der Merwe et al., 1994). These measurements validated early speculation that adhesion molecules would self-assemble into a highly ordered interface in which interactions would be confined to $\sim 1 \text{ nm}$ deep volume (confinement length) between the interacting cells (Bell, 1978). This is not the space between the cell membranes, but the space in which the binding sites of the receptors and ligands encounter each other. Computer simulations of cadherins, another family of adhesion molecules, also support the importance of confinement for 2D affinity (Wu et al., 2013, Wu et al., 2011). The photobleaching studies suggest that effective dwell time of the receptor and ligand is at least 100-fold longer than the zero-force binding half-live in solution, which has implications for signaling by receptor with fast off- and on-rates (Aleksic et al., 2010).

Similar approaches to 2D affinity calculations have been applied in the more complex environment of the immunological synapse. The three studies described here all were performed with SLB and focused on the TCR-pMHC interaction, but required ICAM-1 in the SLB, the ligand for LFA-1 on T cells. The measurement of a steady state 2D Kd is possible in the center of the immunological synapse, also referred to by Kupfer and colleagues as the central supramolecular activation complex (cSMAC) because the TCR-MHCp interactions segregate fully from the LFA-1-ICAM-1 interactions and reach a steady-state density after 20-30 minutes. In this context the 2D Kd for a TCR-agonist MHCp interaction was $10 \text{ molecules}/\mu\text{m}^2$ (Grakoui et al., 1999). The estimated confinement length was 1.2 nm , similar to that of CD2-CD58 interaction, as noted above. Photobleaching

recovery experiments in the cSMAC, revealed minimal recovery, consistent with efficient rebinding (Grakoui et al., 1999), also following the precedent set by the CD2-CD58 interaction. TCR in the cSMAC are present in extracellular microvesicles that have no F-actin and can generate no forces (Choudhuri et al., 2014), accounting for the efficient ligand trapping. In contrast, the TCR-pMHC interactions in other regions of the synapse may have different levels of exposure to forces that would keep the system out of equilibrium. Huppa et al further single molecule fluorescence resonance energy transfer to conclude that F-actin dependent forces decreases the half-life of the interaction of TCR with pMHC by ~10-fold, compared to solution interactions, in the periphery of the immune synapse (Huppa et al., 2010). O'Donoghue et al and our unpublished observations demonstrated that TCR can transport single pMHC for much longer times, consistent with lower force exposure or rebinding to TCR after dissociation (O'Donoghue et al., 2013). Regardless, the interactions in the periphery of the immunological synapse, the sites of active signaling, are shorter lived than those in the cSMAC.

Single bond formation and kinetics: adhesion frequency assays

An alternative approach to measure 2D Kd is to focus on the process of forming the first interaction in an interface. The rationale for studying these initial interactions is that there are biological contexts where formation and breakage of single interactions account for the biology, such as rolling adhesion mediated by selectins and some integrins in the vasculature. In addition, every adhesive interface likely starts with one interaction that then either breaks, or is joined by other interactions to develop into the type of actively steered self-assembly described above. One way to measure this is an adhesion frequency assay.

There are a number of configurations, but the assays entirely rely on the ability to indirectly detect single receptor-ligand interactions in an interface based on visualization of microscopically visible sensor. The Zhu group introduced an adhesion frequency assay based on detection of interactions between a nucleated cell bearing a receptor and a red blood cell (RBC) bearing a ligand (Chesla et al., 1998). This approach measures the equilibrium between formation and breakage of small numbers of bonds using visualization of the deformation of a ligand-coated RBC as it is withdrawn from a brief contact with a receptor-bearing cell using micromanipulation with micropipettes. This was initially applied to Fc receptors (Chesla et al., 1998). Hundreds of trials of contact and withdrawal are performed and the probability of tether formation following short periods of defined contact is determined. The ligand density on the RBC is titrated to generate adhesion frequencies of less than 20% to bias towards single interactions. Some adhesion systems show time dependent changes or “memory” and likely result from active remodeling of the non-RBC cell surface to increase or decrease the actual contact area or organization of receptors (Zarnitsyna et al., 2007). In systems that display memory, the adhesion frequency can increase (positive) or decrease (negative) as more trials are performed, and the TCR is reported to display positive memory (Zarnitsyna et al., 2007).

Application of adhesion frequency assays to the TCR recognizing a series of pMHC ligands with different functional effects resulted in the surprising observation that the 2D on-rate is the parameter that best correlates with functional potency (Huang et al., 2010), whereas the

2D off-rate varied little. One uncertainty in these experiments is the effective contact area, which is a relevant parameter in on-rate determination. The apparent contact area is observed based on the microscopy to be a circular area with a diameter of a few μm . However, it is assumed that due to surface roughness of the T cell, the actual contact area is with the ends of projections on the T cell and may only be on the order of 1% of the apparent contact area (Chesla et al., 1998). Not knowing the actual contact area is a significant limitation of this approach, but the notion that the quality of TCR-pMHC interaction can be amplified into large differences in 2D on-rate should stimulate further study to unravel the mechanisms underlying this effect.

Human RBC are used for adhesion frequency assays because they have a low background of interaction with most nucleated cells and they are 'soft', allowing deformation in response to single interactions. However, caution is required; Human RBC used for the aforementioned studies express CD58 and ICAM-4. CD58 binds CD2 on human T cells, but not on mouse T cells (Selvaraj et al., 1987). Most studies to date have been performed with mouse T cells. ICAM-4 is also called the Landsteiner-Weiner blood-group antigen or CD242, which could bind to integrins expressed on T cells such as VLA-4 and LFA-1 (Hermant et al., 2000, Spring et al., 2001) and could potentially contribute to co-stimulatory activity in this system under some conditions. Integrin interactions are divalent cation dependent, and this may be useful way to exclude these, but care must be taken with different cells paired with RBC. The biomembrane force probe (BFP) approach, discussed below, gets around this issue because the ligands are carried on small glass beads attached to the apex of the erythrocyte.

Direct examination of intercellular molecular tethers using a biomembrane force probe

While the adhesion frequency assay allows measurement of single bond formation and breakage kinetics in a static interface, it is unable to query the characteristics of the intercellular molecular tether and its response to external force. The BFP method, developed by Evans and colleagues, goes a step beyond the adhesion frequency assay in that it allows direct examination of the tether dynamics and rupture under different loading forces and rates (Evans and Kinoshita, 2007).

The BFP also uses an RBC as part of the transducer, but links to this cell a small bead that can be imaged with nanometer and millisecond resolution (Evans and Kinoshita, 2007). Importantly, it is predicted that agonist pMHC on a 2 μm diameter glass bead of the type used in the force probe would be on the borderline for T cell stimulation under static conditions due to a surface contact area requirement discussed earlier (Mescher, 1992). This may have facilitated the demonstration of force dependent effects as the bead alone is unlikely to be stimulatory. The different types of pMHC are attached to the bead at a density that forms 0 or 1 interaction per contact cycle with a low percentage of tethers (less than 20%). The micromanipulation of the force probe then allows the single bond tether that forms to be tested by forces at various rates. Thus, the response of the TCR-pMHC bond to different forces and rates of force application could be determined.

Liu et al used the BFP to determine the duration of TCR-pMHC interaction under “0” force or under increasing forces from 5 to 30 pN applied at 1000 pN/s (Liu et al., 2014). Application of a force of 10 pN resulted in a dramatic differentiation of agonist and antagonist ligands. The antagonist ligand interactions are shorter lived, with increased force-reflecting a slip bond behavior in this range. In contrast, agonist ligand interaction become longer lived with increased force- leading to their definition as catch bonds (Figure 1A). As force is further increased the agonists bonds also start to display shorter lifetimes- meaning that even the new molecular configuration that gives rise to the catch bond will eventually be overcome as forces continue to increase. These results suggest that while solution interaction measurements provided some insight into the nature of TCR-pMHC interactions, there are further differences between agonists and weak agonists that can on be revealed by applying external force.

It is important to point out that the measurement of bond lifetime in the setting of “0” force in the BFP experiment is quite different than measurement in solution, which might also be described as “0” force. When ~ 7 nm sized molecules are linked to a live cells that are undergoing high frequency thermal fluctuations at $\pm \sim 1$ pN on a length scale of nanometers, then for “0” force measurement actually exposes the bond to much more external loading that would be experience in solution. Such considerations may also impact the adhesion frequency assay, which could be thought of as “0” force, but in which bonds are subjected to forces generated continually in live cells. Consistent with application of ~1 pN forces to the bonds, all the lifetimes measured by the force probe are shorter than interactions measured in solution (Alam et al., 1999). It is also important to recognize that this system is designed to test the “naked” TCR-pMHC interaction, with no support from adhesion molecule interactions that would normally operate in parallel within an immunological synapse. Therefore, the basal measurements are made under conditions of low oscillating forces where the less potent antagonist ligands actually live longer than the high potency agonist ligands. This contrasts with true “0” force measurement in solution in which the agonist has a longer lifetime than the weakest binding pMHC. This emphasizes the uniqueness of the BFP measurement, and the adhesion frequency assay, compared to any solution measurement, including those made with soluble ligands on live cells (Stepanek et al., 2014).

On-rate vs off-rate

The adhesion frequency and BFP experiments suggest two ways in which agonist pMHC are superior to weaker agonist and antagonist pMHC ligands: higher on-rate in adhesion frequency assays and slower off-rate under increasing force in the BFP experiments. The relationship between these is not clear. The range of on-rate enhancement seen with pMHC in the adhesion frequency assay were dramatic, but seem to represent a quantitative spectrum that spreads the ligands out without making any obvious qualitative distinctions, apart from being able to assign a threshold between the stimulatory and non-stimulatory ligands- there was not distinct break in behavior. In contrast, catch bond formation allows qualitative resolution of agonists (catch bonds at 10 pN) from non-agonists (slip bonds at 10 pN) for mature T cells. A recent study of soluble ectodomains of a human TCR and pMHC variants using hydrodynamic rolling revealed catch bond behavior for only 1 of 7 agonist ligands

tested (Robert et al., 2012). One caveat is that the loading forces were greater than 10 pN, and thus may have been in the slip bond regime for most of the ligands. Nonetheless, this failure to observe catch bonds with soluble fragments of TCR $\alpha\beta$ only also raises the possibility that the holoreceptor, with the extracellular CD3 subunits, may be needed for catch bonds to be observed. The function of the extracellular Ig domains of the immunoreceptor signal transduction subunits is unknown, and regulation of conformation under force as not been tested. It is also conceivable that on-rate enhancement and catch-bond formation by agonists are alternative strategies that operate in the context of immunological synapses.

Forcing functional responses

The Zhu lab is the first group to combine a BFP with a means of measuring cell activation - cytoplasmic Ca^{2+} imaging using a sensitive ratiometric dye (Grynkiewicz et al., 1985). Using this combination they found that T cells can integrate multiple discrete contacts with the BFP to generate a signaling event. T cells that accumulate a total of 10 seconds of “cumulative lifetimes” in a 1 minute period generate a strong Ca^{2+} flux. In contrast, cells that take longer than a minute to accumulate the same duration of catch bonds fail to activate Ca^{2+} signals. This could be the result of hundreds of 0.1 s test cycles with the BFP probe stochastically forming single tethers. Its most likely that different pMHC on the probe and TCR on the T cell were involved in each tether lifetime and its not clear if the signaling is the result of one signaling site (for example, a microcluster of TCR) or the critical signal can be integrated from multiple TCR clusters in the contact footprint of the 2 μm bead. The density of TCR at on microvilli and pMHC on the bead suggest that there are opportunities for different receptors to participate given that there may be contacts with multiple microvilli. The small molecule statistics of the system also likely favor binding events taking place in different locations unless the earlier events favor later events. Prior studies with the TCR-pMHC system in adhesion frequency experiments actually suggest that the TCR has what was referred to as “positive memory” in serial trials- meaning that the probability of generating tethers is increased by prior tethers (Zarnitsyna et al., 2007). Early work had shown that T cells provided with abundant agonist pMHC by photoactivation could generate a Ca^{2+} flux in 6 seconds (Huse et al., 2007). This suggest that the formation of serial TCR-pMHC interactions in a membrane patch over a minute loose little efficiency in terms of forming an effective signaling complex, taking 10 seconds with multiple transient touches (Liu et al., 2014) or 6 seconds through a stable preformed junction with adhesion molecules (Huse et al., 2007). This result has important implications for serial triggering models as it suggest that not only do TCR remember encounters with pMHC over a period of seconds, but that different engaged TCR can share this information to make an activation decision. This result is interesting whether or not the tethers are based on single or multiple TCR-pMHC interactions in each trial.

The profile of the Ca^{2+} response in Liu et al is typical of those elicited by TCR cross-linking in that there is a rapid rise followed by a decrease to an intermediate level (Weiss et al., 1984). When ongoing TCR signals are interrupted the Ca^{2+} levels typically return to baseline after a few minutes (Valitutti et al., 1995, Varma et al., 2006). This profile is even observed with recognition of a single agonist pMHC, although this didn't lead to a sustained

Ca²⁺ increase under static conditions (Irvine et al., 2002). In the example providing by Liu et al, the accumulations of TCR-pMHC catch bonds stops after ~160 seconds, yet the Ca²⁺ level remains above the threshold set for activation for about 140 seconds after the last binding event (Liu et al., 2014). While this experiment was not designed to look at the need for ongoing TCR interactions, and this requirement should be studied in more detail, the single representative observation suggest that T cells maintain signaling for a prolonged period after this mode of stimulation, without further TCR triggering. This is unusual and also raises the possibility that this process may generate additional structures that work to sustain signaling in the absence of visible contact with beads. As mentioned above, the 2 μ m bead with a modest density of pMHC is not expected to trigger any signal in a T cell. It seems likely that a critical threshold in this process may be generating sufficient inositol-3 phosphate to deplete the endoplasmic reticulum of Ca²⁺ and open Ca²⁺ release activated Ca²⁺ (CRAC) channels (Feske et al., 2006).

The BFP allows very fine measurements, but in this case is being interfaced with a very challenging subject- a live T cell that is undergoing a response to activating ligands. Therefore, it is worth considering the nature of the contact interface. As mentioned above for the adhesion frequency approach, the force probe will interact with an irregular surface with many microvilli that can be modeled as small cylinders studding the surface (Mege et al., 1986). Microvilli are near to the resolution limit and other issues of light microscopy near round cells, which are themselves refractile, means that the microvilli cannot be visualized by light microscopy, but will contribute to the apparent cell edge when approaching with the probe bead. So when the bead is pushed against this edge its most likely pushed against the microvilli, particularly in resting cells (Figure 1A,B). The bead may be large enough to contact more than one projection from the cell, some of which can be quite fine and flexible. The formation of a bond with an agonist pMHC will then hold the bead in a position where it may then be in range of other projections such as nanotubes, which can engage in signaling (Chauveau et al., 2010). Whether or not all of these interactions would alter bead position and alert the experimenter is unclear. These caveats will likely be further explored as others adopt these methods.

A parallel study using the adhesion frequency assay with concurrent Ca²⁺ imaging went a step further by showing that the repeated application of force from the RBC enhanced the T cell response over static contact with the RBC (Pryshchep et al., 2014). The challenge with stimulation of T cells with RBC in static conditions is that the RBC is too soft to provide the necessary mechanical feedback (Judokusumo et al., 2012). Pryshchep et al show that stiffening the RBC membrane during application of cyclic contact and withdrawal cycles increased the Ca²⁺ response. The profile of Ca²⁺ elevation was transient at lower membrane tension and more sustained at higher membrane tension in the RBC. These results suggest that dynamics of the APC membrane including extension and retraction of processes, in addition to the average mechanical properties, could impact T cells. The caveat is that the rate of withdrawal by the micropipette may be greater than could be achieved by an antigen-presenting cell.

The signaling memory that is implied by the ability to integrate 10 seconds of signaling over 1 minute suggests a mechanism to avoid dissipation of signaling intermediates. This is

reminiscent of kinetic proof-reading, which is the underpinning of many TCR triggering models (McKeithan, 1995). However, whereas kinetic-proofreading has generally been applied to one receptor that is occupied for some time by a pMHC, it is unlikely that the same TCR is engaged in successive contact-withdrawal cycles so the results suggest that this must be integrated at some higher level. One solution to these problems would be to have the complexes form in a confined space, perhaps something like a dendritic spine in the nervous system. It has been noted that even in situation where MHC-peptide complexes are abundant and uniformly distributed on a surface that T cells interacting with the surface will initiate signaling from a few discrete points and maintain these sites as dominant points of signalosome formation even after the cells have generated large contact areas (Bunnell et al., 2002, Campi et al., 2005). It is assumed that these microclusters correspond to pre-existing projections (microvilli or filipodia) that are abundant on the T cell surface leading to the roughness noted above. The confinement of signaling intermediates in such projections might have a number of effects to promote signaling and actin or membrane curvature based barriers to diffusion in these structures might provide partial protection against dissipative mechanisms. As long as the structure persists through the entire minute needed to integrate signals then this might provide a structural basis for supramolecular memory implied by these results.

Concluding Remarks

The finding that the TCR responds to force via formation of a catch bond opens a new area of investigation leading to a plethora of questions (Box 3). It has been assumed that molecules like CD2 and CD28 would form adhesion domains in which TCR-pMHC interactions are facilitated (Shaw and Dustin, 1997, Springer, 1990, van der Merwe et al., 1995), but would these additional interactions shield the TCR-pMHC bond from forces that are important to test for catch bond character and distinguish agonists from antagonists? Alternatively, it has been suggested that the size mismatch between some adhesion molecules, like LFA-1-ICAM-1, and the TCR-pMHC actually exert force on the TCR to promote deformation (Li et al., 2010, Ma et al., 2008). It is conceivable that the TCR may actually need to segregate from similarly sized adhesion systems in order to maintain optimal exposure to integrin and actin generated forces. These observations suggest that there may be an advantage to spatially isolating the TCR-pMHC from other interactions such that force can be applied. This could be an evolutionary rationale for isolating the TCR in protein islands that prevent co-localization with other receptors on a nanometer length scale (Lillemeier et al., 2010).

Acknowledgments

We thank B. Liu and P. Jönsson for helpful discussions. MLD is supported by a Wellcome Trust Principal Research Fellowship, Kennedy Trust for Rheumatology Senior Research Fellowship and US National Institutes of Health grant AI043542.

References

1. Alam SM, Davies GM, Lin CM, Zal T, Nasholds W, Jameson SC, Travers PJ, et al. Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands. *Immunity*. 1999; 10:227–237. [PubMed: 10072075]

2. Aleksic M, Dushek O, Zhang H, Shenderov E, Chen JL, Cerundolo V, van der Merwe PA, et al. Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. *Immunity*. 2010; 32:163–174. [PubMed: 20137987]
3. Bashour KT, Gondarenko A, Chen H, Shen K, Liu X, Huse M, Kam LC, et al. CD28 and CD3 have complementary roles in T-cell traction forces. *Proc Natl Acad Sci U S A*. 2014
4. Bell GI. Models for the specific adhesion of cells to cells. *Science*. 1978; 200:618–627. [PubMed: 347575]
5. Bunnell SC, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, Barr VA, Samelson LE. T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. *J Cell Biol*. 2002; 158:1263–1275. [PubMed: 12356870]
6. Campi G, Varma R, Dustin ML. Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. *J Exp Med*. 2005; 202:1031–1036. [PubMed: 16216891]
7. Chauveau A, Aucher A, Eissmann P, Vivier E, Davis DM. Membrane nanotubes facilitate long-distance interactions between natural killer cells and target cells. *Proc Natl Acad Sci U S A*. 2010; 107:5545–5550. [PubMed: 20212116]
8. Chesla SE, Selvaraj P, Zhu C. Measuring two-dimensional receptor-ligand binding kinetics by micropipette. *Biophys J*. 1998; 75:1553–1572. [PubMed: 9726957]
9. Choudhuri K, Llodra J, Roth EW, Tsai J, Gordo S, Wucherpfennig KW, Dustin ML, et al. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature*. 2014; 507:118–123. [PubMed: 24487619]
10. Daniels MA, Teixeira E, Gill J, Hausmann B, Roubaty D, Holmberg K, Palmer E, et al. Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature*. 2006; 444:724–729. [PubMed: 17086201]
11. Dembo M, Torney DC, Saxman K, Hammer D. The reaction-limited kinetics of membrane-to-surface adhesion and detachment. *Proceedings of the Royal Society of London Series B, Containing papers of a Biological character Royal Society*. 1988; 234:55–83.
12. Dustin ML, Bromley SK, Davis MM, Zhu C. Identification of self through two-dimensional chemistry and synapses. *Annu Rev Cell Dev Biol*. 2001; 17:133–157. [PubMed: 11687486]
13. Dustin ML, Ferguson LM, Chan PY, Springer TA, Golan DE. Visualization of CD2 interaction with LFA-3 and determination of the two-dimensional dissociation constant for adhesion receptors in a contact area. *J Cell Biol*. 1996; 132:465–474. [PubMed: 8636222]
14. Dustin ML, Starr T, Coombs D, Majeau GR, Meier W, Hochman PS, Whitty A, et al. Quantification and Modeling of Tripartite CD2-, CD58FC Chimera (Alefcept)-, and CD16-mediated Cell Adhesion. *J Biol Chem*. 2007; 282:34748–34757. [PubMed: 17911103]
15. Evans E, Kinoshita K. Using force to probe single-molecule receptor-cytoskeletal anchoring beneath the surface of a living cell. *Methods Cell Biol*. 2007; 83:373–396. [PubMed: 17613317]
16. Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Rao A, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006; 441:179–185. [PubMed: 16582901]
17. Galbraith CG, Yamada KM, Sheetz MP. The relationship between force and focal complex development. *J Cell Biol*. 2002; 159:695–705. [PubMed: 12446745]
18. Garcia KC, Tallquist MD, Pease LR, Brunmark A, Scott CA, Degano M, Teyton L, et al. Alphabeta T cell receptor interactions with syngeneic and allogeneic ligands: affinity measurements and crystallization. *Proc Natl Acad Sci U S A*. 1997; 94:13838–13843. [PubMed: 9391114]
19. Govern CC, Paczosa MK, Chakraborty AK, Huseby ES. Fast on-rates allow short dwell time ligands to activate T cells. *Proc Natl Acad Sci U S A*. 2010; 107:8724–8729. [PubMed: 20421471]
20. Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: A molecular machine controlling T cell activation. *Science*. 1999; 285:221–227. [PubMed: 10398592]
21. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem*. 1985; 260:3440–3450. [PubMed: 3838314]
22. Hermard P, Huet M, Callebaut I, Gane P, Ihanus E, Gahmberg CG, Bailly P, et al. Binding sites of leukocyte beta 2 integrins (LFA-1, Mac-1) on the human ICAM-4/LW blood group protein. *J Biol Chem*. 2000; 275:26002–26010. [PubMed: 10846180]

23. Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N, Evavold BD, Zhu C. The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. *Nature*. 2010; 464:932–936. [PubMed: 20357766]
24. Huppa JB, Axmann M, Mortelmaier MA, Lillemeier BF, Newell EW, Brameshuber M, Davis MM, et al. TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. *Nature*. 2010; 463:963–967. [PubMed: 20164930]
25. Huse M, Klein LO, Girvin AT, Faraj JM, Li QJ, Kuhns MS, Davis MM. Spatial and temporal dynamics of T cell receptor signaling with a photoactivatable agonist. *Immunity*. 2007; 27:76–88. [PubMed: 17629516]
26. Ilani T, Vasiliver-Shamis G, Vardhana S, Bretscher A, Dustin ML. T cell antigen receptor signaling and immunological synapse stability require myosin IIA. *Nat Immunol*. 2009; 10:531–539. [PubMed: 19349987]
27. Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM. Direct observation of ligand recognition by T cells. *Nature*. 2002; 419:845–849. [PubMed: 12397360]
28. Jonsson P, McColl J, Clarke RW, Ostanin VP, Jonsson B, Klenerman D. Hydrodynamic trapping of molecules in lipid bilayers. *Proc Natl Acad Sci U S A*. 2012; 109:10328–10333. [PubMed: 22699491]
29. Judokusumo E, Tabdanov E, Kumari S, Dustin ML, Kam LC. Mechanosensing in T lymphocyte activation. *Biophys J*. 2012; 102:L5–7. [PubMed: 22339876]
30. Kersh GJ, Kersh EN, Fremont DH, Allen PM. High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. *Immunity*. 1998; 9:817–826. [PubMed: 9881972]
31. Kim ST, Takeuchi K, Sun ZY, Touma M, Castro CE, Fahmy A, Reinherz EL, et al. The alphabeta T cell receptor is an anisotropic mechanosensor. *J Biol Chem*. 2009; 284:31028–31037. [PubMed: 19755427]
32. Li YC, Chen BM, Wu PC, Cheng TL, Kao LS, Tao MH, Roffler SR, et al. Cutting Edge: mechanical forces acting on T cells immobilized via the TCR complex can trigger TCR signaling. *J Immunol*. 2010; 184:5959–5963. [PubMed: 20435924]
33. Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM. TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. *Nat Immunol*. 2010; 11:90–96. [PubMed: 20010844]
34. Liu B, Chen W, Evavold BD, Zhu C. Accumulation of Dynamic Catch Bonds between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell*. 2014; 157:357–368. [PubMed: 24725404]
35. Ma Z, Janmey PA, Finkel TH. The receptor deformation model of TCR triggering. *FASEB J*. 2008; 22:1002–1008. [PubMed: 17984179]
36. Matsui K, Boniface JJ, Steffner P, Reay PA, Davis MM. Kinetics of T-cell receptor binding to peptide/I-Ek complexes: correlation of the dissociation rate with T-cell responsiveness. *Proc Natl Acad Sci U S A*. 1994; 91:12862–12866. [PubMed: 7809136]
37. McKeithan TW. Kinetic proofreading in T-cell receptor signal transduction. *Proc Natl Acad Sci U S A*. 1995; 92:5042–5046. [PubMed: 7761445]
38. Mege JL, Capo C, Benoliel AM, Foa C, Galindo R, Bongrand P. Quantification of cell surface roughness; a method for studying cell mechanical and adhesive properties. *J Theor Biol*. 1986; 119:147–160. [PubMed: 3488469]
39. Mescher MF. Surface contact requirements for activation of cytotoxic T lymphocytes. *J Immunol*. 1992; 149:2402–2405. [PubMed: 1527386]
40. Negulescu PA, Krasieva TB, Khan A, Kerschbaum HH, Cahalan MD. Polarity of T cell shape, motility, and sensitivity to antigen. *Immunity*. 1996; 4:421–430. [PubMed: 8630728]
41. O'Connor RS, Hao X, Shen K, Bashour K, Akimova T, Hancock WW, Milone MC, et al. Substrate rigidity regulates human T cell activation and proliferation. *J Immunol*. 2012; 189:1330–1339. [PubMed: 22732590]
42. O'Donoghue GP, Pielak RM, Smoligovets AA, Lin JJ, Groves JT. Direct single molecule measurement of TCR triggering by agonist pMHC in living primary T cells. *eLife*. 2013; 2:e00778. [PubMed: 23840928]

43. Pryshchep S, Zarnitsyna VI, Hong J, Evavold BD, Zhu C. Accumulation of Serial Forces on TCR and CD8 Frequently Applied by Agonist Antigenic Peptides Embedded in MHC Molecules Triggers Calcium in T Cells. *J Immunol.* 2014; 193:68–76. [PubMed: 24890718]
44. Qi S, Krogsgaard M, Davis MM, Chakraborty AK. Molecular flexibility can influence the stimulatory ability of receptor-ligand interactions at cell-cell junctions. *Proc Natl Acad Sci U S A.* 2006; 103:4416–4421. [PubMed: 16537380]
45. Robert P, Aleksic M, Dushek O, Cerundolo V, Bongrand P, van der Merwe PA. Kinetics and mechanics of two-dimensional interactions between T cell receptors and different activating ligands. *Biophys J.* 2012; 102:248–257. [PubMed: 22339861]
46. Selvaraj P, Plunkett ML, Dustin M, Sanders ME, Shaw S, Springer TA. The T lymphocyte glycoprotein CD2 (LFA-2/T11/E-Rosette receptor) binds the cell surface ligand LFA-3. *Nature.* 1987; 326:400–403. [PubMed: 2951597]
47. Shaw AS, Dustin ML. Making the T cell receptor go the distance: a topological view of T cell activation. *Immunity.* 1997; 6:361–369. [PubMed: 9133415]
48. Spring FA, Parsons SF, Ortlepp S, Olsson ML, Sessions R, Brady RL, Anstee DJ. Intercellular adhesion molecule-4 binds alpha(4)beta(1) and alpha(V)-family integrins through novel integrin-binding mechanisms. *Blood.* 2001; 98:458–466. [PubMed: 11435317]
49. Springer TA. Adhesion receptors of the immune system. *Nature.* 1990; 346:425–434. [PubMed: 1974032]
50. Stepanek O, Prabhakar AS, Osswald C, King CG, Bulek A, Naeher D, Palmer E, et al. Coreceptor scanning by the T cell receptor provides a mechanism for T cell tolerance. *Cell.* 2014; 159:333–345. [PubMed: 25284152]
51. Thomas W, Forero M, Yakovenko O, Nilsson L, Vicini P, Sokurenko E, Vogel V. Catch-bond model derived from allostery explains force-activated bacterial adhesion. *Biophys J.* 2006; 90:753–764. [PubMed: 16272438]
52. Tolentino TP, Wu J, Zarnitsyna VI, Fang Y, Dustin ML, Zhu C. Measuring diffusion and binding kinetics by contact area FRAP. *Biophys J.* 2008; 95:920–930. [PubMed: 18390627]
53. Valitutti S, Dessing M, Aktories K, Gallati H, Lanzavecchia A. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. *J Exp Med.* 1995; 181:577–584. [PubMed: 7836913]
54. van der Merwe PA, Barclay AN, Mason DW, Davies EA, Morgan BP, Tone M, Davis SJ, et al. Human cell-adhesion molecule CD2 binds CD58 (LFA-3) with a very low affinity and an extremely fast dissociation rate but does not bind CD48 or CD59. *Biochemistry.* 1994; 33:10149–10160. [PubMed: 7520278]
55. van der Merwe PA, McNamee PN, Davies EA, Barclay AN, Davis SJ. Topology of the CD2-CD48 cell-adhesion molecule complex: implications for antigen recognition by T cells. *Curr Biol.* 1995; 5:74–84. [PubMed: 7697352]
56. Varma R, Campi G, Yokosuka T, Saito T, Dustin ML. T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. *Immunity.* 2006; 25:117–127. [PubMed: 16860761]
57. Weiss A, Imboden J, Shoback D, Stobo J. Role of T3 surface molecules in human T-cell activation: T3-dependent activation results in an increase in cytoplasmic free calcium. *Proc Natl Acad Sci U S A.* 1984; 81:4169–4173. [PubMed: 6234599]
58. Wu Y, Honig B, Ben-Shaul A. Theory and simulations of adhesion receptor dimerization on membrane surfaces. *Biophys J.* 2013; 104:1221–1229. [PubMed: 23528081]
59. Wu Y, Vendome J, Shapiro L, Ben-Shaul A, Honig B. Transforming binding affinities from three dimensions to two with application to cadherin clustering. *Nature.* 2011; 475:510–513. [PubMed: 21796210]
60. Xie J, Huppa JB, Newell EW, Huang J, Ebert PJ, Li QJ, Davis MM. Photocrosslinkable pMHC monomers stain T cells specifically and cause ligand-bound TCRs to be ‘preferentially’ transported to the cSMAC. *Nat Immunol.* 2012; 13:674–680. [PubMed: 22660579]
61. Zarnitsyna VI, Huang J, Zhang F, Chien YH, Leckband D, Zhu C. Memory in receptor-ligand-mediated cell adhesion. *Proc Natl Acad Sci U S A.* 2007; 104:18037–18042. [PubMed: 17991779]

Glossary Box

Adhesion memory

a concept in the adhesion frequency assay in which the proportion of successful trials increases (positive memory) or decrease (negative memory) as more trials are performed on a given cell.

Bond

in the context of cell-cell adhesion a bond is typically a non-covalent interaction between surface anchored molecules that forms a bridge between the two surfaces. Single or multiple bonds mediate adhesion and different types of bonds often operate in parallel.

Catch bond

a bond in which the off-rate decreases as force is applied. Note that the minimal off-rate is achieved at a specific force and loading rate and further increases in force applied will eventually convert the catch bond to a slip bond leading to bond rupture.

Kinetic rate constants

the rates for bond formation (k_{on} , on-rate, volume/molecules*time) and bond dissociation (k_{off} , off-rate, 1/time) at equilibrium. These rates describe probabilities of bond formation and a distribution of lifetimes at the level of signal molecules or pairs of molecules.

Slip bond

a bond in which the off-rate increases as force is applied until bond rupture.

Three-dimensional (solution) dissociation constant (3D K_d)

is an intrinsic property of an interaction determine through the law of mass action. For an interaction of A and B- $3D K_d = ([A]*[B]) / [AB]$ at equilibrium. Units are molecules/volume. This is the inverse of affinity.

Two-dimensional dissociation constant (2D K_d)

a phenomenological parameter related to the interaction of molecules in the interface between two surfaces at equilibrium. Defined by Bell (1978)- $3D K_d = 2D K_d / \sigma$ in which σ is defined as the confinement length in units of length. Units of 2D K_d are molecules/area.

Box 1**The catch bond**

The molecular mechanism by which the T cell receptor displays catch bond behavior is not known. However, one conceptual mechanism is based on a force induced allosteric change that generates additional interactions that delay the slip bond behavior.

Figure I.

A. A slip bond with the potential to form only one interaction that is destabilized by applied force. B. Force exerted by the blue molecule upfolds one of the domains to expose a second binding site complementary to a site on the green ligand. The additional binding energy stabilizes the bond, but as force is increased both of these bonds eventually fail.

Box 3**Outstanding Questions**

1. What is the force map of the immunological synapse?
2. Is the TCR or the pMHC (or both, or neither) the force sensor?
3. Do MHC class I and class II restricted TCR differ in force requirements?
4. Can polyvalency substitute for force in TCR triggering? E.g.- how does antibody mediated cross-linking activate T cells?
5. How does an immunological synapse accelerate Ca^{2+} signaling compared to single molecule triggering.
6. Do allosteric changes associated with the catch bond also change the conformation of the CD3 cytoplasmic domains to initiate signaling?
7. Do other immunoreceptors, costimulators and checkpoint regulators operate by force-induced allostery?
8. Do small adhesion molecules like CD2-CD58 and other SLAM family members shield the TCR-pMHC interaction from force?
9. Do T cells sense tissue stiffness and respond to modify TCR signals and cellular responses?
10. What are the implications of force sensing for design of chimeric antigen receptors used in immunotherapy?

Highlights

- Recent studies have examined how TCR-pMHC interaction responds to force
- The TCR uses a catch bond that responds to certain force thresholds
- This mechanism may enable discrimination of specific antigens
- Other interactions in the immunological synapse may impact TCR catch bond formation

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Figure 1.

Biomembrane force probe experiment. (A) A 2 μm glass bead is affixed to an RBC held by a stationary micropipet. Oriented pMHC are linked to the bead through a streptavidin-biotin interaction. A live T cell held by a separate micropipette that can be moved with nm precision is docked to the bead to generate a small contact area, probably with microvilli studding the lymphocyte surface. The T cell profile is traced from a thin section transmission electron micrograph of a non-polarized lymphoblast. Microvilli cannot be resolved by light microscopy, but the averaged refractility of all the microvilli is detected and the bead is brought into firm contact with this boundary (B). At 10x higher magnification it is imagined that the cell-bead contact slightly deforms the microvilli and generates a contact area that is about 2% of the apparent contact area. (C) At the densities used for agonist pMHC there is a 10-20% chance that a single TCR-pMHC tether will form across all the microvilli (μvilli). So when the T cell is retracted after a 0.1 s touch there is a 10-20% chance that a tether will be detected based on damping of oscillations in the bead. The tether can then be positioned to allow the tether be exposed only to the BFP oscillations of ± 1 pN or be loaded with forces of up to 30 pN at a rate of 1000 pN/s. Holding the force at 10 pN was the optimal conditions to observe catch-bond behavior in agonist pMHC.