HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

J Immunol. 2023 August 01; 211(3): 333–342. doi:10.4049/jimmunol.2300141.

What's the Catch? – The Significance of Catch Bonds in T cell Activation

Michael A. Faust[*],

Viva J. Rasé[*],

Tracey J. Lamb,

Brian D. Evavold

Division of Microbiology and Immunology, Department of Pathology, University of Utah, Salt Lake City, UT 84112

Abstract

One of the main goals in T cell biology has been to investigate how T cell receptor (TCR) recognition of peptide:MHC (pMHC) antigens determines T cell phenotype and fate. Antigen recognition is required to facilitate survival, expansion, and effector function of T cells. Historically TCR affinity for pMHC has been used as predictor for T cell fate and responsiveness, but there have now been several examples of nonfunctional high affinity clones and low affinity highly functional clones. Recently, more attention has been paid to the TCR being a mechanoreceptor where the key biophysical determinant is TCR bond lifetime under force. As outlined in this review, the fundamental parameters between the TCR and pMHC that control antigen recognition and T cell triggering are affinity, bond lifetime, and the amount of force at which the peak lifetime occurs.

Introduction – T cell Interaction with Antigen

The nature of the adaptive immune response largely depends on a variety of antigen-specific T cell effector functions such as help, inflammation, targeted cell cytotoxicity, or suppression. At its core, T cell activation is initiated when the T cell receptor (TCR) engages an appropriate peptide antigen presented by major histocompatibility complex (pMHC). Ultimately, this response outcome depends on intracellular signaling triggered by the TCR after engagement with pMHC although biological factors including various cytokines, surface molecules, and small biomolecules can influence final T cell phenotype and function. The extent of intracellular signaling derives from the kinetics of T cell receptor interaction with the pMHC ligand (1–3). Typically, receptor interactions with ligand are determined at equilibrium with overall affinity used to distinguish ligands of different potency. However, analysis of the T cell receptor as well as many other receptors has begun to introduce the idea of active processes at the cell surface that may alter the time that a

Corresponding author: Brian Evavold, brian.evavold@path.utah.edu, (801) 585-9689.
[*]Indicates authors contributed equally.

Conflict of Interest: The authors declare no conflict of interest.

receptor interacts with its ligand (4–13). Thus, the kinetic parameters that define the TCR's interaction with pMHC determine the phenotype of adaptive immunity. A noncovalent protein bond typically refers to specific atomic interactions such as hydrogen bonds, salt bridges, or van der Waals forces; however, in this review TCR:pMHC bond refers to the collection of atomic interactions between TCR and pMHC regardless of their specific identity or composition.

The TCR is tasked with translating antigenic properties into functional outputs, arguably one of the most difficult jobs of any cell surface receptor. It has a complex ligand comprised of a peptide fragment presented by a self-derived MHC molecule. This results in several inherent operational constraints to this interaction. First, the TCR must trigger sufficient levels of signals through its interaction with self-peptide MHC for survival during thymic selection (14–16). This remains true in the periphery, where tonic signaling must exist yet remain below the threshold that would otherwise trigger autoimmunity (17, 18). When responding to foreign antigen, the TCR must also be sensitive enough to differentiate between a diverse array of functional responses to antigens that may differ by only a single amino acid and are scarcely expressed amongst thousands of total pMHC on each target APC. This probably explains why a T cell can become activated by as few as a single pMHC molecule and why TCRs naturally possess some level of cross-reactivity with multiple antigens since there is unlikely to be enough independent TCRs expressed in any individual to respond to each possible 20 amino acid variation in a 9-mer peptide fragment. Given this set of operating parameters, the TCR cannot merely be a bimodal switch for activation. Instead, recent advances have revealed a finely tuned mechanosensor complex that successfully translates subtle differences in antigen into intracellular signals.

The Rise and Fall of Affinity

TCR affinity for pMHC is often thought to be the driving factor for the level of T cell response (2, 3, 19–22). While a TCR must have sufficient affinity for cognate pMHC ligands to form a bond, affinity does not always effectively correlate with response. Originally, the limitation in affinity measurements occurred because many of the initial studies of T cell antigen recognition utilized soluble TCR and pMHC proteins. This meant that purified, non-native proteins were analyzed in three-dimensional (3D) space as opposed to the two-dimensional (2D) interactions that occur between membrane bond receptors and their ligands. Moreover, the TCR is not an isolated molecule - it is noncovalently associated with CD3 proteins embedded at the interface between two cells, and the inclusion of CD3 will alter TCR kinetics. Anomalies became apparent in studies wherein the 3D affinities did not match T cell functional outcomes. Examples of this are seen in low affinity TCRs that respond to ligation (23–27) or high affinity TCRs that are unresponsive to ligation (7, 28). Sometimes, a complete lack of correlation between TCR:pMHC affinity and T cell function was observed, especially with respect to altered peptide ligands (APLs) (26, 29–32). Kinetic off-rates reported in 3D at times improved functional outcomes, but even this readout does not accurately explain all T cell functional response (23, 33–35). Therefore, since both TCR and pMHC are membrane-bound and molecularly complex, it comes as little surprise that studying their kinetics in 2D space gives a more accurate correlation with biological response (2, 3, 36).

In 1996, peptide-loaded MHC tetramers became an important tool for working with antigen-reactive T cells (37). Tetramers consist of streptavidin:biotin linkages of four pMHC molecules to provide sufficient avidity for binding and quantification via flow cytometry. Identifying T cells with peptide-MHC tetramers placed an unintentional focus on higher affinity TCRs. However, in many cases, antigen-responsive T cells with lower affinity TCRs that could not bind tetramers were identified (38–45). Several of these studies have shown that low affinity T cells are equally as responsive as high affinity responders (41–45). In autoimmune demyelinating disease, depletion of high affinity tetramer-positive T cells using CAR T cells bearing pMHC chimeric antigen receptors does not fully ameliorate ongoing demyelinating disease (46), further implying that T cells baring low-affinity TCRs effectively contribute to disease (41). Thus, it has become increasingly clear that affinity could not exclusively describe the T cell response.

TCR Mechanosensing

A T cell is in motion while searching for antigen (47–49), naturally subjecting any surface interaction to shear and tensile forces. Using a 2D-micropipette based system (3), we have visualized the effects of antigen recognition on T cell spreading over time using CD8 T cells obtained from mice infected with LCMV for 8 days. The still-shot frames (Figure 1) and included movie demonstrate that force can be applied to the TCR:pMHC bond (and presumably any other intermolecular bonds on the cell surface subsequent to TCR engagement). Initially, a CD8 T cell (Figure 1A, **left cell**) is brought into contact with a red blood cell (RBC) coated with pMHC (Figure 1A, **right cell**). As time passes, the T cell morphs by sending out projections in order to sample antigen on the target cell (Figure 1B). After a few minutes, the T cell pulls the pMHC-coated RBC from the opposing pipette (Figure 1C). Since the RBC is solely coated with pMHC, Video 1 provides evidence that pMHC alone is capable of triggering a TCR-driven hunt for antigen by the T cell. This also demonstrates the dynamic interface between T cell and APC. The observed crawling by the T cell imposes physical stress to the collective TCR:pMHC bonds, which is especially apparent as the T cell pulls the RBC from the opposing micropipette. When the TCR was described as a mechanosensor by Reinherz and colleagues (4), several labs began to dissect how physical stress in the form of force applied to the TCR:pMHC interaction affects the duration of the TCR:pMHC bond and in turn how the changes in bond lifetime impacts T cell biology (5–13).

T cells are highly mobile and mechanosensing offers a unique mechanism to explain the anomalies presented by APLs given the motile nature of T cells within their environment. Mechanosensing is the ability of a membrane-bound receptor to respond to mechanical stress, namely force, applied with an indicated directionality. Several types of bond lifetimes exist for mechanosensing proteins interacting with their ligands. These can include: (1) slip bonds, the decrease in bond lifetimes under increasing mechanical force; (2) catch-slip bonds, wherein bond lifetimes increase under increasing mechanical force before reaching a maximal bond lifetime (catch), after which increasing force yields decreasing bond lifetimes (slip); (3) or ideal bonds, which are insensitive to mechanical force. P-selectin was the first identified immunological receptor that forms catch bonds with its ligand (50). Since then,

a collection of immunological catch bond-forming receptors has been identified (Table I), including the TCR:pMHC interaction (4, 5, 8).

Using anti-CD3 activating antibodies, the TCR was first described as an anisotropic mechanosensor varying in the magnitude of antigen detection according to the direction of force (4). The mechanosensing abilities of the TCR have since been validated by a collection of techniques (Table I). Notably, the biomembrane force probe measures the bond lifetimes of single TCR:pMHC interactions (5). The TCR:pMHC catch bond occurs up to a particular amount of force, usually ~10 pN (5–8, 11–13), at which point the peak bond lifetime is observed. Most techniques read out the bond lifetimes under applied force. However, T cell application of force can be visualized using DNA tension sensor probes which consist of pMHC monomers conjugated to fluorescently-labelled double-stranded DNA-based tension sensors that become unraveled as a T cell applies force (73, 74). Importantly, TCR strength of signal, measured by the phosphorylation of intracellular signaling molecule Zap70, discussed below, has now been shown to be directly related to ligand potency (73, 74) providing a biological context to T cells displaying different bond lifetimes under force.

Correlation of 2D Affinity with Bond Lifetime Under Force

It is likely that for many ligands the affinity measured under 2D conditions and peak bond lifetime under force will correlate with the greatest T cell effector functions Figure 2A (5, 6, 8, 10, 13, 75–77). That implies a lower affinity antigen will equate to a lower bond lifetime under force while a higher affinity antigen equates to a longer bond lifetime under force (3, 5, 6). It should be noted that the 2D range in affinity for polyclonal TCRs can span 4-logs with the sensitivity to measure positive-selecting and antagonist ligands (38, 42, 78, 79). Of this broad affinity range, pMHC tetramers identify only the upper portion, although some recent advances in reengineered tetramers may increase the overall detectable range (80). The reported bond lifetimes cover a smaller 2-log range of 0.1–10 seconds at 10 pN for agonists (3, 5–7, 77). This level of force is independent of both the MHC allele used and the presence of coreceptor. To date, less potent APLs can display lower affinity, peak bond lifetimes, and the amount of force at which the peak lifetime is found (5, 77). A peak bond lifetime that occurs greater than 2x that of 10 pN has yet to be reported. Collectively, this implies that 10 pN identifies the optimal level of force for T cell effector functions and brings about the question of how effector responses are modulated by bond lifetime under force.

Outlier TCR:pMHC Interactions

As more TCR:pMHC ligand interactions are reported, we will be able to further define how often the affinity and bond lifetime under force coordinate. However, the rare outlier TCRs that fall off of the line in the extreme quadrants of the graph will also be of importance in understanding T cell activation to antigen (Fig 2A). For example, several high affinity TCRs as identified by pMHC tetramer, 3D solution affinity, and/or 2D affinity fail to trigger T cells on encounter with antigen (7, 28). This surprising finding of high affinity TCRs that were incapable of functional responses indicates that when bond lifetime and affinity are disparate, bond lifetime under force determines the outcome. Some high affinity, nonresponsive TCRs were found to bind in a reversed orientation that likely impairs

signaling (28). In these cases, the catch bond may be acting as quality control for the fidelity of the TCR. The use of catch bond lifetimes to vet antigens is likely to be important given the random nature of TCR, VDJ recombination, and the possibility to generate receptors that engage pMHC incorrectly or even bind other proteins (12).

Additional examples where bond lifetime under force likely dominates over affinity occur in autoimmune or tumor responses. The autoimmune 2D2 TCR is specific for a myelin antigen and mediates a very well-established model of autoimmune demyelinating disease (81). Despite the 2D2 TCR being derived from a mouse that can spontaneously develop disease, its affinity for MOG is below detection using the 2D micropipette system or pMHC tetramer (82). A strong relationship between lifetime and response would predict that 2D2 might possess a reasonable bond lifetime disparate from its extremely low affinity. Similarly, there may be low affinity TCRs that are effective against self-tumor antigens if their bond lifetime is sufficient to trigger effector functions (13, 29, 40, 76, 83).

Although the analysis of TCR:pMHC bond lifetimes is in its early stages, it is expected to find additional ligands that deviate from the direct correlation between affinity and catch bond lifetimes. In each of these cases, the catch bond is expected to be the driving parameter for response. Another example where bond lifetime under force rather than affinity predicts the outcome of T cell response rather than affinity is in the OT1 OVA altered peptide ligand system (APL). The use of wild type peptide OVA (SIINFEKL N4) is a stronger TCR agonist than its APL A2 (alanine substituted in the second position) (33, 84). Intriguingly, the 2D affinity measurements between the two peptides are comparable (3) and thus affinity by itself is not accurate in predicting the level of response in this case. Peak bond lifetime falls at 10 pN of force for both N4 and A2 but the lifetime of the N4MHC:TCR bond is about twice as long as A2MHC:TCR bond (5). Thus, A2 is a weaker agonist because of its reduced bond lifetime.

Modified Antigen for Increased Bond Lifetime

The molecular mechanism(s) of a catch bond are continuing to be defined. Molecular modeling with applied force has proven informative on what may occur when force is applied to a TCR:pMHC bond (7, 13, 85). Such analysis can suggest mutations that may alter the binding kinetics at the TCR interface with antigen. For example, a TCR (Figure 2A, purple symbol) specific for an HIV peptide $pol_{448–456}$ (TCR55) failed to form a productive catch bond under increasing forces and thus did not trigger T cells despite being identified through a pMHC tetramer screen and possessing high affinity (7). Instead, the kinetics for the TCR55 bond were an example of a slip bond. To achieve T cell triggering and functional responses, TCR55 was screened with mutant peptide antigen libraries. Several high-affinity variant HIV peptide antigens gained the capacity to form catch bonds with TCR55 leading to functional T cell responses. Since this initial TCR had high affinity for antigen, this study served as proof of concept that catch bonds could be altered independently of affinity (7).

Modified TCR for Increased Bond Lifetime

Often APLs are used to successfully modify T cell responses, with the different functional outcomes referred to as changes in the affinity between TCR and the APL. This relationship

to affinity is very likely a misconception since the variant antigens generally alter the amount of force and the peak bond lifetime under force in addition to affinity (5, 6, 12, 77). The multiple effects likely result from making amino acid changes at the interface of the peptide and the CDR3 regions of TCR. In these cases, it is difficult to determine which of the parameters causes the change in strength of signal from the resulting APL. The results generated using the modified antigen discussed above indicated that it may be plausible to maintain equal or lower affinity and engineer select functions in T cells through altering bond lifetime under force. Alternatively, it may also be possible to target the distal amino acids of the TCR CDR3 regions with the goal of altering bond lifetimes under force with minimal effects on affinity (Figure 2B). Among several single amino acid mutations in the HIV-specific TCR55, an alanine to histidine mutation in the CDR3 resulted in a 3-fold lower affinity for antigen yet remarkably a gain in T cell function (76). Unsurprisingly, this mutated version of TCR55 formed catch bonds under force, continuing to demonstrate the relationship between response and catch bonds (76). This also validates the process of engineering the TCR protein to affect the characteristics of the resulting bond lifetime under force with antigen.

Often tumor-specific TCRs are identified with low affinity for the self-antigen. This observation biased the anti-tumor strategy towards increasing the equilibrium affinity of the TCR to more effectively attack the tumor cells. Previously a wild type low affinity MAGE-A3 tumor antigen TCR was identified but it was not highly responsive to antigen. A high affinity TCR called A3A was derived from wild type MAGE A3 TCR through affinity maturation. T cells containing these high affinity A3A TCRs were extremely responsive to MAGE-A3 antigens and used in clinical trials. Unfortunately, A3A CD8 T cells engaged in unpredicted off target cytotoxicity of cardiac cells expressing a cross-reactive epitope in the Titin protein (86). The results demonstrated that TCRs engineered for high affinity to tumor antigens might lead to more off target and unintended detrimental side effects targeting of cross-reactive antigens on healthy non-tumor cells (86, 87). In addition, the data suggests that greatly increased equilibrium affinity may be the incorrect parameter to focus on to improve tumor antigen specific-TCRs. The CDR3 regions of the original wild type low affinity anti-tumor MAGE-A3 TCR were mutated with the goal to increase effector efficacy via increased bond lifetimes under force while maintaining the original affinity (Figure 2B) and presumably the same level of cross-reactivity for other self-antigens. Several potential CDR3 mutations were found that maintained the original TCR affinity to MAGE-A3 tumor antigen yet led to increased levels of functional response. Two of the modified TCRs were analyzed for bond lifetime under force and were found to form strong catch bonds. The TCR with the longest bond lifetime proved to be the most potent effector and killer cell. Importantly, these modified TCRs were unresponsive to the previous cross-reactive cardiac Titin antigen. In line with relationship between responsiveness and catch bond lifetime under force, the TCRs were also screened for bond lifetime to the cross-reactive Titin antigen. Of note, the original high affinity and deadly A3A TCR was the only TCR to form a strong catch bond (76).

Key Contributors to TCR:pMHC Catch Bonds

Mechanoreceptors and catch bond kinetics can be directly influenced by the local environment, the developmental history of the cells, the composition of the membrane, and the expression level of several co-localizing surface proteins. In the case of T cell triggering, the protein players include the TCR, the pMHC ligand, CD3 proteins, the CD4/8 coreceptors, Lck associated with coreceptor, T cell intracellular signaling complex, and the actin cytoskeleton. Together, each contributes to the maximal bond lifetime under force. To date, most of the focus has been on the effects of peptide antigen on bond lifetime with more recent analysis of the TCR and co-receptor. A major impetus for the identification of TCR:pMHC catch bond effects was the use of APLs as a surrogate of ligands of different potency to probe T cell responses (5). The single amino acid changes of most APLs can generate the entire spectrum of pharmacologic ligands including agonists, partial agonists, and antagonists. From some of the work on TCR outlined above, it is apparent that TCR protein sequence and structure can also influence the affinity and bond lifetime of antigen recognition (28, 76). It would be of no surprise if MHC stability including epitope affinity for MHC and MHC structural integrity also influence the nature of the bond (catch vs slip) or its kinetics (bond lifetime under force). Ongoing work continues to refine the respective roles of peptide:MHC antigen and TCR in the catch bond phenotype.

Coreceptor Force Contribution

In addition to TCR engaging its cognate pMHC, coreceptor plays a role in enhancing T cell activation. In fact, blocking antibodies targeting coreceptor are commonly used to thwart T cell function. It is known that coreceptor enhances the trimolecular interaction and later the culmination of intracellular signaling, yet the mechanism of this under 2D constraints is only just becoming clear. The CD8αβ heterodimer binds MHC via the α subunit with low 2D affinity relative to TCR (11, 36, 88). Importantly, the trimolecular interaction occurs in a stepwise manner whereby TCR engages pMHC before CD8 enhances the interaction (11, 36). Recent evidence suggests that CD4 also behaves in a similar stepwise mechanism, albeit the affinity of CD4 for MHC class II is very low (80, 89, 90). Our lab first demonstrated that coreceptor can contribute as much as a 15-fold increase in bond lifetime under force using a CD8 TCR model with viral variant peptides (6). Since then, it’s been shown that coreceptor enhances the TCR:pMHC:coreceptor trimolecular interaction by increasing bond lifetime or changing the force at which peak bond lifetime occurs (6, 77, 89, 91). Thymic selection is also affected by coreceptor as CD8 T cells are positively selected for shorter lived slip bonds whereas negative selection occurs via catch bonds under force by the TCR:pMHC:CD8 coreceptor complex (12). As measured by DNA tension sensor, CD8 coreceptor is essential for T cell mediated force generation and T cell spreading (73). Given the biological importance of coreceptor engagement during T cell activation, understanding the mechanism by which the co-receptor modulates catch bond formation will provide additional key information on T cell activation.

TCR Strength of Signal

TCR signal strength is a key driver of T cell fate and function. While often invoked to describe differential outcomes for T cells, the definition of signal strength in terms of

the kinetics involved between TCR and pMHC is not well defined, be it affinity or bond lifetime under force or more likely some combination of both. TCR strength of signal is often reported using surrogate markers downstream of the TCR:pMHC interaction such as Nur77 (92–94), IRF4 (95), CD5 (96), and more recently activation induced markers (CD69, CD25, CD44, and OX40) (97–99). Historically, APLs have been used to assess affinity and functional outcome (3, 84, 100) but undoubtably APLs alter bond lifetime under force (5, 6, 13). This is apparent in the OTI system where it was demonstrated using SIINFEKL APLs that TCR agonists (N4, A2, G2) formed catch bonds and antagonists (R4 and E1) formed slip bonds (5). In addition, these same APLs have been used to show that Ca2+ signaling requires bond lifetime under force (5). Recently, it was found that TCR signal strength can be tuned through formation of force mediated engineered catch bonds (76). Importantly, this study correlated CD69 expression with bond lifetime under force rather than affinity (76).

Innate T cells/TCR Interactions

Almost all of the published work on TCR lifetime under force has focused on traditional αβ receptors from cells of the adaptive response. Additional information of the mechanosensing restrictions on the TCR can be garnered by considering innate T cells, such as γδ T, NKT, and MAIT cells. Of these T cell subsets, a single γδ TCR/ligand interaction has been reported (101) whereby, the γδ TCR was unable to form catch bonds with an activating ligand sulfatide presented by CD1d. This γδ TCR could be engineered for catch bond characteristics through the insertion of the constant region from a traditional αβ TCR. While this supports the notion of αβ structural integrity being a key component to the ability of TCRs to form catch bonds, it is also possible that catchable ligands for the γδ TCR have yet to be analyzed. Additional analysis of other ligands for γδ TCRs would determine whether the lack of catch bond features is a general characteristic of all γδ TCR/ligand interactions. The use of catch bonds under force by iNKT and MAIT cells to distinguish ligands is unknown at this time. Invariant natural killer T (iNKT) cells express a fixed α-chain with limited β-chains that recognize glycolipids presented by CD1d. Although the proposed TCR:CD1d binding orientation on engagement with αGalCer:CD1d differs from that of TCR engaging with peptide antigens, the αβ TCR is structurally similar to canonical CD8/CD4 T cell TCRs which may support catch bond behavior. Similarly, mucosal-associated invariant T (MAIT) cells also express a semi-invariant αβ TCR that recognizes vitamin B-derived ligands presented by the non-classical MR1 MHC molecule. Analysis of each of these innate T cell subtypes would further inform on the relationship of TCR structure, catch-bond behavior, and effector function.

TCR:pMHC Catch Bond Initiates a Positive Feedback Signaling Loop

Here, we summarize the respective players that transpose antigen recognition into response for naïve T cells. The first interactions between APCs and T cells are determined by TCR affinity for pMHC (Figure 3A and 3B) (2, 3, 22, 84, 87, 102–106). Following the initial TCR:pMHC interaction that happens at zero force, coreceptor rapidly joins the complex (36, 84, 90). This begins the process of assessment of the quality of the interaction through the resulting bond lifetime via the application of force on the bond. Intra- and extracellular forces initiate the positive feedback signaling cascade that tests bond lifetime under increasing force magnitude (Figure 3C) (4, 107). As the TCR itself

does not contain cytosolic signaling domains, it associates non-covalently with CD3 made up of two heterodimers, γε and δε, and one ζζ homodimer, which signal via a total of 10 immunoreceptor tyrosine biased activation motif (ITAMs) phosphorylation sites (83, 108–111). Indeed, full activation of the T cell requires association of the CD3 complex and allosteric conformational changes (4, 112, 113). The CD4/CD8 coreceptors further potentiate TCR:pMHC interaction by bringing activated lymphocyte specific tyrosine kinase (LCK) into contact with ITAMs. LCK engages and phosphorylates the ITAMs (28) (Figure 3C). This facilitates the recruitment of zeta chain associated kinase 70 (ZAP70) to the ITAMs (114) (Figure 3D). ZAP70 aids in phosphorylation of linker of activated T cells (LAT), which serves as a hub for the transduction of TCR initiated signaling events (115, 116). This initial signal propagation occurs while TCR:pMHC are in the bound state and is enhanced by molecular forces initiating a positive feedback loop (36) of effector signaling events. Canonically this is known as inside out signaling since cytosolic changes result in heightened TCR:pMHC or other extracellular interactions (12, 117–119) (Figure 3D). As TCR signaling is amplified, we expect that the force applied on the TCR:pMHC bonds increase (denoted by increasing gear size over time in Figure 3). For example, longer bond lifetime under increased force aids in the dynamic rearrangement of the actin cytoskeleton (120–122) initiated by VAV1 a cytoplasmic guanine nucleotide exchange factor for Rho family GTPases (123). Several different actin inhibitors have been shown to prevent T cell spreading and TCR force generation indicating the importance of actin retrograde flow in TCR inside out signaling (73).

An additional effect of the increased signaling are changes in integrins. The integrins are also dynamic proteins responsive to force that increase their level of interaction through catch bond formation. Integrins change to an open conformation under force that has been detected by interferometric photoactivation and localization microscopy. In short, when integrins such as lymphocyte function associated antigen 1 (LFA-1) are in the inactivated form, a fluorophore attached is quenched; but, when LFA-1 enters in the open state bound to intercellular adhesion molecule 1 (ICAM-1) under force this fluorophore is visible by fluorescent microscope (124, 125). Interestingly, LFA-1:ICAM-1 bonds under force have been shown to enhance TCR:pMHC bonds (126). Throughout the course of an immunological synapse between T cell and APC, TCR:pMHC bonds will reach maximal force which results in the bond breaking completing the test of the antigen quality. TCR signaling propels the reorientation of the cytosolic tails of costimulatory molecule CD28 optimizing PI3K signaling (127). In addition to costimulation, check point inhibitors such as PD-1 can directly alter bond lifetime (128). It is unclear to the field whether TCRs can undergo serial engagement, however, undoubtably new TCR:pMHC bonds under force form (3, 8, 129) (Figure 3E).

Conclusions

Ultimately, the TCR is an example of a mechanosensor influenced by several proteins to accomplish the task of translating antigen quality into strength of signal controlling T cell fate and phenotype. This is accomplished through the application of force to the TCR:pMHC bond to discern the potency of the antigen. Future studies manipulating the structure of the TCR will uncover key contact points for efficient outside/in signaling

while disentangling the relationship between affinity, bond lifetime, and force. Beyond the intricacies of αβTCR, additional study of innate T cells and non-classical MHCs will further inform on the many possible triggers of T cell responses. Regardless of innate or adaptive TCRs, there are many biological contexts remaining to be to examined to define how the kinetics of antigen recognition determine response. Of note, the 2D kinetic measurements can be applied to all areas of T cell biology. In thymic selection, the preTCR has been identified as a mechanosensor that interacts more strongly with antigen than the mature TCR (72). During later steps of thymic selection, catch bonds are required for thymocyte death during negative selection whereas survival of positive selection was dependent on slip bond formation (12). In other words, bond lifetime under force supports a mechanism of thymic selection whereby T cells must interact strongly enough with self to survive (positive selection) but will be deleted if these interactions are too strong to avoid host-directed immune responses (negative selection). After selection, in the periphery, continuous sampling of self-antigen is required to provide tonic survival signals, yet the onset of autoimmune disease is rare. It is of interest to determine what kinetic level allows for these tonic signals from self-peptides for survival; however, at this time many of the self-epitopes mediating this process are unknown (130). Similarly, T cell mediated autoimmunity is commonly associated with specific MHC alleles. As the kinetics of self-antigen recognition for these alleles are discovered, we may finally be able to dissect their role in the progression of autoimmune disease. Understanding the biological implications of changes to bond lifetime under force will allow for targeted manipulation of the kinetics of the TCR to potentially generate more effective Tregs, preferred effector phenotypes, or enhanced CAR T cells. Such engineered TCRs or chimeric antigen receptors (CARs) targeting both cancer and autoimmune epitopes have been developed (9, 46). Bond lifetime under force may be more informative as opposed to affinity when designing such adoptive transfer therapies. In a similar light, pMHC-CAR technology can be used for deletion of autoreactive T cells (46). Bond lifetime under force may increase their efficacy as well, which could include the screening of APLs or enhancement of coreceptor contribution (80). Thus, the TCR as a mechanosensor and how the affinity, type of bond (slip vs catch), and bond lifetime under force probe the unique features of antigen will finally provide the insight and understanding of determine of T cell activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Grants:

This research was supported in part by the National Institutes of Health (T32NS115664, T32AI148945, R01NS071518, R01AI169835, and R01AI167422).

REFERNCES

1. Kane LP, Lin J, and Weiss A. 2000. Signal transduction by the TCR for antigen. Curr Opin Immunol 12: 242–249. [PubMed: 10781399]
2. Huppa JB, Axmann M, Mortelmaier MA, Lillemeier BF, Newell EW, Brameshuber M, Klein LO, Schutz GJ, and Davis MM. 2010. TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. Nature 463: 963–967. [PubMed: 20164930]

3. Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N, Evavold BD, and Zhu C. 2010. The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. Nature 464: 932–936. [PubMed: 20357766]
4. Kim ST, Takeuchi K, Sun ZY, Touma M, Castro CE, Fahmy A, Lang MJ, Wagner G, and Reinherz EL. 2009. The alphabeta T cell receptor is an anisotropic mechanosensor. J Biol Chem 284: 31028–31037. [PubMed: 19755427]
5. Liu B, Chen W, Evavold BD, and Zhu C. 2014. Accumulation of dynamic catch bonds between TCR and agonist peptide-MHC triggers T cell signaling. Cell 157: 357–368. [PubMed: 24725404]
6. Kolawole EM, Andargachew R, Liu B, Jacobs JR, and Evavold BD. 2018. 2D Kinetic Analysis of TCR and CD8 Coreceptor for LCMV GP33 Epitopes. Front Immunol 9: 2348. [PubMed: 30374353]
7. Sibener LV, Fernandes RA, Kolawole EM, Carbone CB, Liu F, McAffee D, Birnbaum ME, Yang X, Su LF, Yu W, Dong S, Gee MH, Jude KM, Davis MM, Groves JT, Goddard WA 3rd, Heath JR, Evavold BD, Vale RD, and Garcia KC. 2018. Isolation of a Structural Mechanism for Uncoupling T Cell Receptor Signaling from Peptide-MHC Binding. Cell 174: 672–687 e627. [PubMed: 30053426]
8. Feng Y, Brazin KN, Kobayashi E, Mallis RJ, Reinherz EL, and Lang MJ. 2017. Mechanosensing drives acuity of alphabeta T-cell recognition. Proc Natl Acad Sci U S A 114: E8204–E8213. [PubMed: 28811364]
9. Banik D, Hamidinia M, Brzostek J, Wu L, Stephens HM, MacAry PA, Reinherz EL, Gascoigne NRJ, and Lang MJ. 2021. Single Molecule Force Spectroscopy Reveals Distinctions in Key Biophysical Parameters of alphabeta T-Cell Receptors Compared with Chimeric Antigen Receptors Directed at the Same Ligand. J Phys Chem Lett 12: 7566–7573. [PubMed: 34347491]
10. Das DK, Feng Y, Mallis RJ, Li X, Keskin DB, Hussey RE, Brady SK, Wang JH, Wagner G, Reinherz EL, and Lang MJ. 2015. Force-dependent transition in the T-cell receptor beta-subunit allosterically regulates peptide discrimination and pMHC bond lifetime. Proc Natl Acad Sci U S A 112: 1517–1522. [PubMed: 25605925]
11. Liu B, Zhong S, Malecek K, Johnson LA, Rosenberg SA, Zhu C, and Krogsgaard M. 2014. 2D TCR-pMHC-CD8 kinetics determines T-cell responses in a self-antigen-specific TCR system. Eur J Immunol 44: 239–250. [PubMed: 24114747]
12. Hong J, Ge C, Jothikumar P, Yuan Z, Liu B, Bai K, Li K, Rittase W, Shinzawa M, Zhang Y, Palin A, Love P, Yu X, Salaita K, Evavold BD, Singer A, and Zhu C. 2018. A TCR mechanotransduction signaling loop induces negative selection in the thymus. Nat Immunol 19: 1379–1390. [PubMed: 30420628]
13. Wu P, Zhang T, Liu B, Fei P, Cui L, Qin R, Zhu H, Yao D, Martinez RJ, Hu W, An C, Zhang Y, Liu J, Shi J, Fan J, Yin W, Sun J, Zhou C, Zeng X, Xu C, Wang J, Evavold BD, Zhu C, Chen W, and Lou J. 2019. Mechano-regulation of Peptide-MHC Class I Conformations Determines TCR Antigen Recognition. Mol Cell 73: 1015–1027 e1017. [PubMed: 30711376]
14. Irla M 2022. Instructive Cues of Thymic T Cell Selection. Annu Rev Immunol 40: 95–119. [PubMed: 35471838]
15. Stritesky GL, Xing Y, Erickson JR, Kalekar LA, Wang X, Mueller DL, Jameson SC, and Hogquist KA. 2013. Murine thymic selection quantified using a unique method to capture deleted T cells. Proc Natl Acad Sci U S A 110: 4679–4684. [PubMed: 23487759]
16. Bendelac A, Matzinger P, Seder RA, Paul WE, and Schwartz RH. 1992. Activation events during thymic selection. J Exp Med 175: 731–742. [PubMed: 1740662]
17. Myers DR, Zikherman J, and Roose JP. 2017. Tonic Signals: Why Do Lymphocytes Bother? Trends Immunol 38: 844–857. [PubMed: 28754596]
18. Eggert J, and Au-Yeung BB. 2021. Functional heterogeneity and adaptation of naive T cells in response to tonic TCR signals. Curr Opin Immunol 73: 43–49. [PubMed: 34653787]
19. Gottschalk RA, Hathorn MM, Beuneu H, Corse E, Dustin ML, Altan-Bonnet G, and Allison JP. 2012. Distinct influences of peptide-MHC quality and quantity on in vivo T-cell responses. Proc Natl Acad Sci U S A 109: 881–886. [PubMed: 22223661]

20. Sykulev Y, Brunmark A, Jackson M, Cohen RJ, Peterson PA, and Eisen HN. 1994. Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes. Immunity 1: 15–22. [PubMed: 7889394]
21. Zehn D, Lee SY, and Bevan MJ. 2009. Complete but curtailed T-cell response to very low-affinity antigen. Nature 458: 211–214. [PubMed: 19182777]
22. Skokos D, Shakhar G, Varma R, Waite JC, Cameron TO, Lindquist RL, Schwickert T, Nussenzweig MC, and Dustin ML. 2007. Peptide-MHC potency governs dynamic interactions between T cells and dendritic cells in lymph nodes. Nat Immunol 8: 835–844. [PubMed: 17632517]
23. Rosette C, Werlen G, Daniels MA, Holman PO, Alam SM, Travers PJ, Gascoigne NR, Palmer E, and Jameson SC. 2001. The impact of duration versus extent of TCR occupancy on T cell activation: a revision of the kinetic proofreading model. Immunity 15: 59–70. [PubMed: 11485738]
24. al-Ramadi BK, Jelonek MT, Boyd LF, Margulies DH, and Bothwell AL. 1995. Lack of strict correlation of functional sensitization with the apparent affinity of MHC/peptide complexes for the TCR. J Immunol 155: 662–673. [PubMed: 7541822]
25. Alam SM, Travers PJ, Wung JL, Nasholds W, Redpath S, Jameson SC, and Gascoigne NR. 1996. T-cell-receptor affinity and thymocyte positive selection. Nature 381: 616–620. [PubMed: 8637599]
26. Pettmann J, Huhn A, Abu Shah E, Kutuzov MA, Wilson DB, Dustin ML, Davis SJ, van der Merwe PA, and Dushek O. 2021. The discriminatory power of the T cell receptor. Elife 10.
27. Davey GM, Schober SL, Endrizzi BT, Dutcher AK, Jameson SC, and Hogquist KA. 1998. Preselection thymocytes are more sensitive to T cell receptor stimulation than mature T cells. J Exp Med 188: 1867–1874. [PubMed: 9815264]
28. Zareie P, Szeto C, Farenc C, Gunasinghe SD, Kolawole EM, Nguyen A, Blyth C, Sng XYX, Li J, Jones CM, Fulcher AJ, Jacobs JR, Wei Q, Wojciech L, Petersen J, Gascoigne NRJ, Evavold BD, Gaus K, Gras S, Rossjohn J, and La Gruta NL. 2021. Canonical T cell receptor docking on peptide-MHC is essential for T cell signaling. Science 372.
29. McMahan RH, McWilliams JA, Jordan KR, Dow SW, Wilson DB, and Slansky JE. 2006. Relating TCR-peptide-MHC affinity to immunogenicity for the design of tumor vaccines. J Clin Invest 116: 2543–2551. [PubMed: 16932807]
30. Anderton SM, Radu CG, Lowrey PA, Ward ES, and Wraith DC. 2001. Negative selection during the peripheral immune response to antigen. J Exp Med 193: 1–11. [PubMed: 11136816]
31. Kersh GJ, Kersh EN, Fremont DH, and Allen PM. 1998. High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. Immunity 9: 817–826. [PubMed: 9881972]
32. Richard AC, Lun ATL, Lau WWY, Gottgens B, Marioni JC, and Griffiths GM. 2018. T cell cytolytic capacity is independent of initial stimulation strength. Nat Immunol 19: 849–858. [PubMed: 30013148]
33. Alam SM, Davies GM, Lin CM, Zal T, Nasholds W, Jameson SC, Hogquist KA, Gascoigne NR, and Travers PJ. 1999. Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands. Immunity 10: 227–237. [PubMed: 10072075]
34. Adams JJ, Narayanan S, Liu B, Birnbaum ME, Kruse AC, Bowerman NA, Chen W, Levin AM, Connolly JM, Zhu C, Kranz DM, and Garcia KC. 2011. T cell receptor signaling is limited by docking geometry to peptide-major histocompatibility complex. Immunity 35: 681–693. [PubMed: 22101157]
35. Zhong S, Malecek K, Johnson LA, Yu Z, Vega-Saenz de Miera E, Darvishian F, McGary K, Huang K, Boyer J, Corse E, Shao Y, Rosenberg SA, Restifo NP, Osman I, and Krogsgaard M. 2013. T-cell receptor affinity and avidity defines antitumor response and autoimmunity in T-cell immunotherapy. Proc Natl Acad Sci U S A 110: 6973–6978. [PubMed: 23576742]
36. Jiang N, Huang J, Edwards LJ, Liu B, Zhang Y, Beal CD, Evavold BD, and Zhu C. 2011. Two-stage cooperative T cell receptor-peptide major histocompatibility complex-CD8 trimolecular interactions amplify antigen discrimination. Immunity 34: 13–23. [PubMed: 21256056]

37. Altman JD, Moss PA, Goulder PJ, Barouch DH, McHeyzer-Williams MG, Bell JI, McMichael AJ, and Davis MM. 1996. Phenotypic analysis of antigen-specific T lymphocytes. Science 274: 94–96. [PubMed: 8810254]
38. Krummey SM, Martinez RJ, Andargachew R, Liu D, Wagener M, Kohlmeier JE, Evavold BD, Larsen CP, and Ford ML. 2016. Low-Affinity Memory CD8+ T Cells Mediate Robust Heterologous Immunity. J Immunol 196: 2838–2846. [PubMed: 26864034]
39. Liu B, Hood JD, Kolawole EM, Woodruff DM, Vignali DA, Bettini M, and Evavold BD. 2020. A Hybrid Insulin Epitope Maintains High 2D Affinity for Diabetogenic T Cells in the Periphery. Diabetes 69: 381–391. [PubMed: 31806623]
40. Rius C, Attaf M, Tungatt K, Bianchi V, Legut M, Bovay A, Donia M, Thor Straten P, Peakman M, Svane IM, Ott S, Connor T, Szomolay B, Dolton G, and Sewell AK. 2018. Peptide-MHC Class I Tetramers Can Fail To Detect Relevant Functional T Cell Clonotypes and Underestimate Antigen-Reactive T Cell Populations. J Immunol 200: 2263–2279. [PubMed: 29483360]
41. Martinez RJ, Andargachew R, Martinez HA, and Evavold BD. 2016. Low-affinity CD4+ T cells are major responders in the primary immune response. Nat Commun 7: 13848. [PubMed: 27976744]
42. Sabatino JJ Jr., Huang J, Zhu C, and Evavold BD. 2011. High prevalence of low affinity peptide-MHC II tetramer-negative effectors during polyclonal CD4+ T cell responses. J Exp Med 208: 81–90. [PubMed: 21220453]
43. Dougan SK, Dougan M, Kim J, Turner JA, Ogata S, Cho HI, Jaenisch R, Celis E, and Ploegh HL. 2013. Transnuclear TRP1-specific CD8 T cells with high or low affinity TCRs show equivalent antitumor activity. Cancer Immunol Res 1: 99–111. [PubMed: 24459675]
44. Tungatt K, Bianchi V, Crowther MD, Powell WE, Schauenburg AJ, Trimby A, Donia M, Miles JJ, Holland CJ, Cole DK, Godkin AJ, Peakman M, Straten PT, Svane IM, Sewell AK, and Dolton G. 2015. Antibody stabilization of peptide-MHC multimers reveals functional T cells bearing extremely low-affinity TCRs. J Immunol 194: 463–474. [PubMed: 25452566]
45. Huang J, Zeng X, Sigal N, Lund PJ, Su LF, Huang H, Chien YH, and Davis MM. 2016. Detection, phenotyping, and quantification of antigen-specific T cells using a peptide-MHC dodecamer. Proc Natl Acad Sci U S A 113: E1890–1897. [PubMed: 26979955]
46. Yi J, Miller AT, Archambault AS, Jones AJ, Bradstreet TR, Bandla S, Hsu YS, Edelson BT, Zhou YW, Fremont DH, Egawa T, Singh N, Wu GF, and Hsieh CS. 2022. Antigen-specific depletion of CD4(+) T cells by CAR T cells reveals distinct roles of higher- and lower-affinity TCRs during autoimmunity. Sci Immunol 7: eabo0777.
47. Henrickson SE, Mempel TR, Mazo IB, Liu B, Artyomov MN, Zheng H, Peixoto A, Flynn M, Senman B, Junt T, Wong HC, Chakraborty AK, and von Andrian UH. 2008. In vivo imaging of T cell priming. Sci Signal 1: pt2.
48. Hickman HD, Takeda K, Skon CN, Murray FR, Hensley SE, Loomis J, Barber GN, Bennink JR, and Yewdell JW. 2008. Direct priming of antiviral CD8+ T cells in the peripheral interfollicular region of lymph nodes. Nat Immunol 9: 155–165. [PubMed: 18193049]
49. Miller MJ, Wei SH, Parker I, and Cahalan MD. 2002. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. Science 296: 1869–1873. [PubMed: 12016203]
50. Marshall BT, Long M, Piper JW, Yago T, McEver RP, and Zhu C. 2003. Direct observation of catch bonds involving cell-adhesion molecules. Nature 423: 190–193. [PubMed: 12736689]
51. Sarangapani KK, Yago T, Klopocki AG, Lawrence MB, Fieger CB, Rosen SD, McEver RP, and Zhu C. 2004. Low force decelerates L-selectin dissociation from P-selectin glycoprotein ligand-1 and endoglycan. J Biol Chem 279: 2291–2298. [PubMed: 14573602]
52. Sarangapani KK, Qian J, Chen W, Zarnitsyna VI, Mehta P, Yago T, McEver RP, and Zhu C. 2011. Regulation of catch bonds by rate of force application. J Biol Chem 286: 32749–32761. [PubMed: 21775439]
53. Klopocki AG, Yago T, Mehta P, Yang J, Wu T, Leppanen A, Bovin NV, Cummings RD, Zhu C, and McEver RP. 2008. Replacing a lectin domain residue in L-selectin enhances binding to P-selectin glycoprotein ligand-1 but not to 6-sulfo-sialyl Lewis x. J Biol Chem 283: 11493–11500. [PubMed: 18250165]

54. Yago T, Wu J, Wey CD, Klopocki AG, Zhu C, and McEver RP. 2004. Catch bonds govern adhesion through L-selectin at threshold shear. J Cell Biol 166: 913–923. [PubMed: 15364963]
55. Liu Z, Yago T, Zhang N, Panicker SR, Wang Y, Yao L, Mehta-D'souza P, Xia L, Zhu C, and McEver RP. 2017. L-selectin mechanochemistry restricts neutrophil priming in vivo. Nat Commun 8: 15196. [PubMed: 28497779]
56. Lou J, Yago T, Klopocki AG, Mehta P, Chen W, Zarnitsyna VI, Bovin NV, Zhu C, and McEver RP. 2006. Flow-enhanced adhesion regulated by a selectin interdomain hinge. J Cell Biol 174: 1107–1117. [PubMed: 17000883]
57. Morikis VA, Chase S, Wun T, Chaikof EL, Magnani JL, and Simon SI. 2017. Selectin catch-bonds mechanotransduce integrin activation and neutrophil arrest on inflamed endothelium under shear flow. Blood 130: 2101–2110. [PubMed: 28811304]
58. Wayman AM, Chen W, McEver RP, and Zhu C. 2010. Triphasic force dependence of E-selectin/ligand dissociation governs cell rolling under flow. Biophys J 99: 1166–1174. [PubMed: 20713000]
59. Evans E, Leung A, Heinrich V, and Zhu C. 2004. Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. Proc Natl Acad Sci U S A 101: 11281–11286. [PubMed: 15277675]
60. Barbaux S, Poirier O, Pincet F, Hermand P, Tiret L, and Deterre P. 2010. The adhesion mediated by the P-selectin P-selectin glycoprotein ligand-1 (PSGL-1) couple is stronger for shorter PSGL-1 variants. J Leukoc Biol 87: 727–734. [PubMed: 20051472]
61. Snook JH, and Guilford WH. 2010. The Effects of Load on E-Selectin Bond Rupture and Bond Formation. Cell Mol Bioeng 3: 128–138. [PubMed: 20526425]
62. Kong F, Garcia AJ, Mould AP, Humphries MJ, and Zhu C. 2009. Demonstration of catch bonds between an integrin and its ligand. J Cell Biol 185: 1275–1284. [PubMed: 19564406]
63. Chen W, Lou J, Evans EA, and Zhu C. 2012. Observing force-regulated conformational changes and ligand dissociation from a single integrin on cells. J Cell Biol 199: 497–512. [PubMed: 23109670]
64. Chen W, Lou J, and Zhu C. 2010. Forcing switch from short- to intermediate- and long-lived states of the alphaA domain generates LFA-1/ICAM-1 catch bonds. J Biol Chem 285: 35967–35978. [PubMed: 20819952]
65. Rosetti F, Chen Y, Sen M, Thayer E, Azcutia V, Herter JM, Luscinskas FW, Cullere X, Zhu C, and Mayadas TN. 2015. A Lupus-Associated Mac-1 Variant Has Defects in Integrin Allostery and Interaction with Ligands under Force. Cell Rep 10: 1655–1664. [PubMed: 25772353]
66. Choi YI, Duke-Cohan JS, Chen W, Liu B, Rossy J, Tabarin T, Ju L, Gui J, Gaus K, Zhu C, and Reinherz EL. 2014. Dynamic control of beta1 integrin adhesion by the plexinD1-sema3E axis. Proc Natl Acad Sci U S A 111: 379–384. [PubMed: 24344262]
67. Buckley CD, Tan J, Anderson KL, Hanein D, Volkmann N, Weis WI, Nelson WJ, and Dunn AR. 2014. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. Science 346: 1254211.
68. Wang A, Dunn AR, and Weis WI. 2022. Mechanism of the cadherin-catenin F-actin catch bond interaction. Elife 11.
69. Rakshit S, Zhang Y, Manibog K, Shafraz O, and Sivasankar S. 2012. Ideal, catch, and slip bonds in cadherin adhesion. Proc Natl Acad Sci U S A 109: 18815–18820. [PubMed: 23112161]
70. Luca VC, Kim BC, Ge C, Kakuda S, Wu D, Roein-Peikar M, Haltiwanger RS, Zhu C, Ha T, and Garcia KC. 2017. Notch-Jagged complex structure implicates a catch bond in tuning ligand sensitivity. Science 355: 1320–1324. [PubMed: 28254785]
71. Bano F, Banerji S, Howarth M, Jackson DG, and Richter RP. 2016. A single molecule assay to probe monovalent and multivalent bonds between hyaluronan and its key leukocyte receptor CD44 under force. Sci Rep 6: 34176. [PubMed: 27679982]
72. Das DK, Mallis RJ, Duke-Cohan JS, Hussey RE, Tetteh PW, Hilton M, Wagner G, Lang MJ, and Reinherz EL. 2016. Pre-T Cell Receptors (Pre-TCRs) Leverage Vbeta Complementarity Determining Regions (CDRs) and Hydrophobic Patch in Mechanosensing Thymic Self-ligands. J Biol Chem 291: 25292–25305. [PubMed: 27707880]

73. Liu Y, Blanchfield L, Ma VP, Andargachew R, Galior K, Liu Z, Evavold B, and Salaita K. 2016. DNA-based nanoparticle tension sensors reveal that T-cell receptors transmit defined pN forces to their antigens for enhanced fidelity. Proc Natl Acad Sci U S A 113: 5610–5615. [PubMed: 27140637]
74. Ma R, Kellner AV, Hu Y, Deal BR, Blanchard AT, and Salaita K. 2021. DNA Tension Probes to Map the Transient Piconewton Receptor Forces by Immune Cells. J Vis Exp.
75. Liu B, Kolawole EM, and Evavold BD. 2021. Mechanobiology of T Cell Activation: To Catch a Bond. Annu Rev Cell Dev Biol 37: 65–87. [PubMed: 34213954]
76. Zhao X, Kolawole EM, Chan W, Feng Y, Yang X, Gee MH, Jude KM, Sibener LV, Fordyce PM, Germain RN, Evavold BD, and Garcia KC. 2022. Tuning T cell receptor sensitivity through catch bond engineering. Science 376: eabl5282.
77. Hong J, Persaud SP, Horvath S, Allen PM, Evavold BD, and Zhu C. 2015. Force-Regulated In Situ TCR-Peptide-Bound MHC Class II Kinetics Determine Functions of CD4+ T Cells. J Immunol 195: 3557–3564. [PubMed: 26336148]
78. Kersh AE, Edwards LJ, and Evavold BD. 2014. Progression of relapsing-remitting demyelinating disease does not require increased TCR affinity or epitope spread. J Immunol 193: 4429–4438. [PubMed: 25267971]
79. Andargachew R, Martinez RJ, Kolawole EM, and Evavold BD. 2018. CD4 T Cell Affinity Diversity Is Equally Maintained during Acute and Chronic Infection. J Immunol 201: 19–30. [PubMed: 29777029]
80. Dileepan T, Malhotra D, Kotov DI, Kolawole EM, Krueger PD, Evavold BD, and Jenkins MK. 2021. MHC class II tetramers engineered for enhanced binding to CD4 improve detection of antigen-specific T cells. Nat Biotechnol 39: 943–948. [PubMed: 33941928]
81. Bettelli E, Pagany M, Weiner HL, Linington C, Sobel RA, and Kuchroo VK. 2003. Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. J Exp Med 197: 1073–1081. [PubMed: 12732654]
82. Rosenthal KM, Edwards LJ, Sabatino JJ Jr., Hood JD, Wasserman HA, Zhu C, and Evavold BD. 2012. Low 2-dimensional CD4 T cell receptor affinity for myelin sets in motion delayed response kinetics. PLoS One 7: e32562.
83. Susac L, Vuong MT, Thomas C, von Bulow S, O'Brien-Ball C, Santos AM, Fernandes RA, Hummer G, Tampe R, and Davis SJ. 2022. Structure of a fully assembled tumor-specific T cell receptor ligated by pMHC. Cell 185: 3201–3213 e3219. [PubMed: 35985289]
84. Yachi PP, Ampudia J, Zal T, and Gascoigne NR. 2006. Altered peptide ligands induce delayed CD8-T cell receptor interaction--a role for CD8 in distinguishing antigen quality. Immunity 25: 203–211. [PubMed: 16872849]
85. Hwang W, Mallis RJ, Lang MJ, and Reinherz EL. 2020. The alphabetaTCR mechanosensor exploits dynamic ectodomain allostery to optimize its ligand recognition site. Proc Natl Acad Sci U S A 117: 21336–21345. [PubMed: 32796106]
86. Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, Litzky L, Bagg A, Carreno BM, Cimino PJ, Binder-Scholl GK, Smethurst DP, Gerry AB, Pumphrey NJ, Bennett AD, Brewer JE, Dukes J, Harper J, Tayton-Martin HK, Jakobsen BK, Hassan NJ, Kalos M, and June CH. 2013. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood 122: 863–871. [PubMed: 23770775]
87. Baumgaertner P, Schmidt J, Costa-Nunes CM, Bordry N, Guillaume P, Luescher I, Speiser DE, Rufer N, and Hebeisen M. 2022. CD8 T cell function and cross-reactivity explored by stepwise increased peptide-HLA versus TCR affinity. Front Immunol 13: 973986.
88. Huang J, Edwards LJ, Evavold BD, and Zhu C. 2007. Kinetics of MHC-CD8 interaction at the T cell membrane. J Immunol 179: 7653–7662. [PubMed: 18025211]
89. Rushdi MN, Pan V, Li K, Choi HK, Travaglino S, Hong J, Griffitts F, Agnihotri P, Mariuzza RA, Ke Y, and Zhu C. 2022. Cooperative binding of T cell receptor and CD4 to peptide-MHC enhances antigen sensitivity. Nat Commun 13: 7055. [PubMed: 36396644]
90. Xiong Y, Kern P, Chang H, and Reinherz E. 2001. T Cell Receptor Binding to a pMHCII Ligand Is Kinetically Distinct from and Independent of CD4. J Biol Chem 276: 5659–5667. [PubMed: 11106664]

91. Pryshchep S, Zarnitsyna VI, Hong J, Evavold BD, and Zhu C. 2014. 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 193: 68–76. [PubMed: 24890718]

92. Elliot TAE, Jennings EK, Lecky DAJ, Rouvray S, Mackie GM, Scarfe L, Sheriff L, Ono M, Maslowski KM, and Bending D. 2022. Nur77-Tempo mice reveal T cell steady state antigen recognition. Discov Immunol 1: kyac009.

93. Ashouri JF, and Weiss A. 2017. Endogenous Nur77 Is a Specific Indicator of Antigen Receptor Signaling in Human T and B Cells. J Immunol 198: 657–668. [PubMed: 27940659]

94. Moran AE, Holzapfel KL, Xing Y, Cunningham NR, Maltzman JS, Punt J, and Hogquist KA. 2011. T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. J Exp Med 208: 1279–1289. [PubMed: 21606508]

95. Krishnamoorthy V, Kannanganat S, Maienschein-Cline M, Cook SL, Chen J, Bahroos N, Sievert E, Corse E, Chong A, and Sciammas R. 2017. The IRF4 Gene Regulatory Module Functions as a Read-Write Integrator to Dynamically Coordinate T Helper Cell Fate. Immunity 47: 481–497 e487. [PubMed: 28930660]

96. Mandl JN, Monteiro JP, Vrisekoop N, and Germain RN. 2013. T cell-positive selection uses self-ligand binding strength to optimize repertoire recognition of foreign antigens. Immunity 38: 263–274. [PubMed: 23290521]

97. Reiss S, Baxter AE, Cirelli KM, Dan JM, Morou A, Daigneault A, Brassard N, Silvestri G, Routy JP, Havenar-Daughton C, Crotty S, and Kaufmann DE. 2017. Comparative analysis of activation induced marker (AIM) assays for sensitive identification of antigen-specific CD4 T cells. PLoS One 12: e0186998.

98. Dan JM, Lindestam Arlehamn CS, Weiskopf D, da Silva Antunes R, Havenar-Daughton C, Reiss SM, Brigger M, Bothwell M, Sette A, and Crotty S. 2016. A Cytokine-Independent Approach To Identify Antigen-Specific Human Germinal Center T Follicular Helper Cells and Rare Antigen-Specific CD4+ T Cells in Blood. J Immunol 197: 983–993. [PubMed: 27342848]

99. Azcutia V, Bassil R, Herter JM, Engelbertsen D, Newton G, Autio A, Mayadas T, Lichtman AH, Khoury SJ, Parkos CA, Elyaman W, and Luscinskas FW. 2017. Defects in CD4+ T cell LFA-1 integrin-dependent adhesion and proliferation protect Cd47−/− mice from EAE. J Leukoc Biol 101: 493–505. [PubMed: 27965383]

100. Kersh GJ, and Allen PM. 1996. Structural basis for T cell recognition of altered peptide ligands: a single T cell receptor can productively recognize a large continuum of related ligands. J Exp Med 184: 1259–1268. [PubMed: 8879197]

101. Mallis RJ, Duke-Cohan JS, Das DK, Akitsu A, Luoma AM, Banik D, Stephens HM, Tetteh PW, Castro CD, Krahnke S, Hussey RE, Lawney B, Brazin KN, Reche PA, Hwang W, Adams EJ, Lang MJ, and Reinherz EL. 2021. Molecular design of the gammadeltaT cell receptor ectodomain encodes biologically fit ligand recognition in the absence of mechanosensing. Proc Natl Acad Sci U S A 118.

102. Soto CM, Stone JD, Chervin AS, Engels B, Schreiber H, Roy EJ, and Kranz DM. 2013. MHC-class I-restricted CD4 T cells: a nanomolar affinity TCR has improved anti-tumor efficacy in vivo compared to the micromolar wild-type TCR. Cancer Immunol Immunother 62: 359–369. [PubMed: 22926060]

103. Gras S, Chadderton J, Del Campo CM, Farenc C, Wiede F, Josephs TM, Sng XYX, Mirams M, Watson KA, Tiganis T, Quinn KM, Rossjohn J, and La Gruta NL. 2016. Reversed T Cell Receptor Docking on a Major Histocompatibility Class I Complex Limits Involvement in the Immune Response. Immunity 45: 749–760. [PubMed: 27717799]

104. Spear TT, Evavold BD, Baker BM, and Nishimura MI. 2019. Understanding TCR affinity, antigen specificity, and cross-reactivity to improve TCR gene-modified T cells for cancer immunotherapy. Cancer Immunol Immunother 68: 1881–1889. [PubMed: 31595324]

105. Sanecka A, Yoshida N, Kolawole EM, Patel H, Evavold BD, and Frickel EM. 2018. T Cell Receptor-Major Histocompatibility Complex Interaction Strength Defines Trafficking and CD103(+) Memory Status of CD8 T Cells in the Brain. Front Immunol 9: 1290. [PubMed: 29922298]

106. Schmid DA, Irving MB, Posevitz V, Hebeisen M, Posevitz-Fejfar A, Sarria JC, Gomez-Eerland R, Thome M, Schumacher TN, Romero P, Speiser DE, Zoete V, Michielin O, and Rufer N. 2010.

Evidence for a TCR affinity threshold delimiting maximal CD8 T cell function. J Immunol 184: 4936–4946. [PubMed: 20351194]

107. Stephens HM, Brazin KN, Mallis RJ, Feng Y, Banik D, Reinherz EL, and Lang MJ. 2022. Measuring alphabeta T-Cell Receptor-Mediated Mechanosensing Using Optical Tweezers Combined with Fluorescence Imaging. Methods Mol Biol 2478: 727–753. [PubMed: 36063340]
108. Krshnan L, Park S, Im W, Call MJ, and Call ME. 2016. A conserved alphabeta transmembrane interface forms the core of a compact T-cell receptor-CD3 structure within the membrane. Proc Natl Acad Sci U S A 113: E6649–E6658. [PubMed: 27791034]
109. Glassman CR, Parrish HL, Deshpande NR, and Kuhns MS. 2016. The CD4 and CD3deltaepsilon Cytosolic Juxtamembrane Regions Are Proximal within a Compact TCR-CD3-pMHC-CD4 Macrocomplex. J Immunol 196: 4713–4722. [PubMed: 27183595]
110. Dong D, Zheng L, Lin J, Zhang B, Zhu Y, Li N, Xie S, Wang Y, Gao N, and Huang Z. 2019. Structural basis of assembly of the human T cell receptor-CD3 complex. Nature 573: 546–552. [PubMed: 31461748]
111. Pandey PR, Rozycki B, Lipowsky R, and Weikl TR. 2021. Structural variability and concerted motions of the T cell receptor - CD3 complex. Elife 10.
112. Rubin B, Knibiehler M, and Gairin JE. 2007. Allosteric changes in the TCR/CD3 structure upon interaction with extra- or intra-cellular ligands. Scand J Immunol 66: 228–237. [PubMed: 17635800]
113. Mariuzza RA, Agnihotri P, and Orban J. 2020. The structural basis of T-cell receptor (TCR) activation: An enduring enigma. J Biol Chem 295: 914–925. [PubMed: 31848223]
114. Zenner G, Vorherr T, Mustelin T, and Burn P. 1996. Differential and multiple binding of signal transducing molecules to the ITAMs of the TCR-zeta chain. J Cell Biochem 63: 94–103. [PubMed: 8891907]
115. Lo WL, and Weiss A. 2021. Adapting T Cell Receptor Ligand Discrimination Capability via LAT. Front Immunol 12: 673196.
116. Wan-Lin Lo MK, Gabrielle Rizzuto H Atakan Ekiz, Kolawole Elizabeth M., Revelo Monica P., Rakieb Andargachew,, and Li Y.-L. T. Zhongmei, Alexander Marson, Evavold Brian D., Dietmar Zehn & Arthur Weiss. 2023. A single-amino acid substitution in the adaptor LAT accelerates TCR proofreading kinetics and alters T cell selection, maintenance and function. Nat Immunol IN PRESS.
117. Menasche G, Kliche S, Bezman N, and Schraven B. 2007. Regulation of T-cell antigen receptor-mediated inside-out signaling by cytosolic adapter proteins and Rap1 effector molecules. Immunol Rev 218: 82–91. [PubMed: 17624945]
118. Springer TA, and Dustin ML. 2012. Integrin inside-out signaling and the immunological synapse. Curr Opin Cell Biol 24: 107–115. [PubMed: 22129583]
119. Sanchez-Lockhart M, Kim M, and Miller J. 2011. Cutting edge: A role for inside-out signaling in TCR regulation of CD28 ligand binding. J Immunol 187: 5515–5519. [PubMed: 22068237]
120. Aramesh M, Mergenthal S, Issler M, Plochberger B, Weber F, Qin XH, Liska R, Duda GN, Huppa JB, Ries J, Schutz GJ, and Klotzsch E. 2021. Functionalized Bead Assay to Measure Three-dimensional Traction Forces during T-cell Activation. Nano Lett 21: 507–514. [PubMed: 33305952]
121. Blumenthal D, and Burkhardt JK. 2020. Multiple actin networks coordinate mechanotransduction at the immunological synapse. J Cell Biol 219.
122. Wahl A, Dinet C, Dillard P, Nassereddine A, Puech PH, Limozin L, and Sengupta K. 2019. Biphasic mechanosensitivity of T cell receptor-mediated spreading of lymphocytes. Proc Natl Acad Sci U S A 116: 5908–5913. [PubMed: 30850545]
123. Salojin KV, Zhang J, and Delovitch TL. 1999. TCR and CD28 are coupled via ZAP-70 to the activation of the Vav/Rac-1-/PAK-1/p38 MAPK signaling pathway. J Immunol 163: 844–853. [PubMed: 10395678]
124. Moore TI, Aaron J, Chew TL, and Springer TA. 2018. Measuring Integrin Conformational Change on the Cell Surface with Super-Resolution Microscopy. Cell Rep 22: 1903–1912. [PubMed: 29444440]

125. Shtengel G, Galbraith JA, Galbraith CG, Lippincott-Schwartz J, Gillette JM, Manley S, Sougrat R, Waterman CM, Kanchanawong P, Davidson MW, Fetter RD, and Hess HF. 2009. Interferometric fluorescent super-resolution microscopy resolves 3D cellular ultrastructure. Proc Natl Acad Sci U S A 106: 3125–3130. [PubMed: 19202073]

126. Ma VP, Hu Y, Kellner AV, Brockman JM, Velusamy A, Blanchard AT, Evavold BD, Alon R, and Salaita K. 2022. The magnitude of LFA-1/ICAM-1 forces fine-tune TCR-triggered T cell activation. Sci Adv 8: eabg4485.

127. Siokis A, Robert PA, Demetriou P, Dustin ML, and Meyer-Hermann M. 2018. F-Actin-Driven CD28-CD80 Localization in the Immune Synapse. Cell Rep 24: 1151–1162. [PubMed: 30067972]

128. Li K, Yuan Z, Lyu J, Ahn E, Davis SJ, Ahmed R, and Zhu C. 2021. PD-1 suppresses TCR-CD8 cooperativity during T-cell antigen recognition. Nat Commun 12: 2746. [PubMed: 33980853]

129. Beeson C, Rabinowitz J, Tate K, Gutgemann I, Chien YH, Jones PP, Davis MM, and McConnell HM. 1996. Early biochemical signals arise from low affinity TCR-ligand reactions at the cell-cell interface. J Exp Med 184: 777–782. [PubMed: 8760833]

130. Zinzow-Kramer WM, Kolawole EM, Eggert J, Evavold BD, Scharer CD, and Au-Yeung BB. 2022. Strong Basal/Tonic TCR Signals Are Associated with Negative Regulation of Naive CD4(+) T Cells. Immunohorizons 6: 671–683. [PubMed: 36100367]

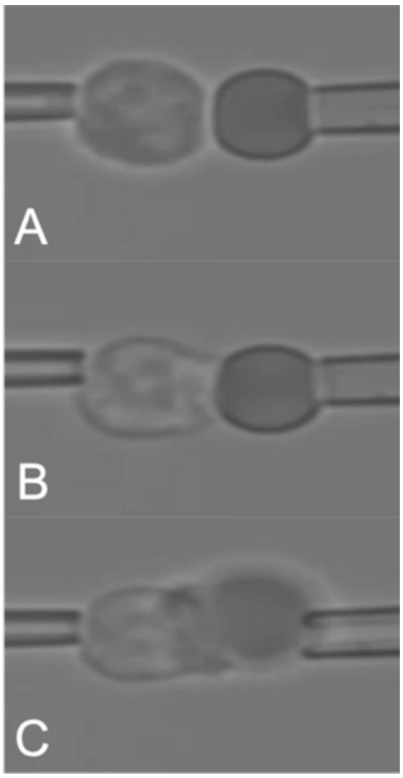

Figure 1: High-affinity T cell lysis of a pMHC-coated target cell -

A splenic CD8 T cell from a C57Bl/6 mouse at peak expansion (day 8) after infection with lymphocytic choriomeningitis virus (LCMV) applies a strong pulling force against cognate pMHC. **A)** After confirming high affinity on 2D-MP, the T cell was manually aligned against a human RBC artificially coated with gp33:H2-D^b. **B)** After two minutes, a significant morphological change is observed in the T cell as it samples antigen. C) After only four minutes, the CD8 T cell pulls the RBC from the opposing pipette and lyses the RBC, indicated by the translucency of the RBC.

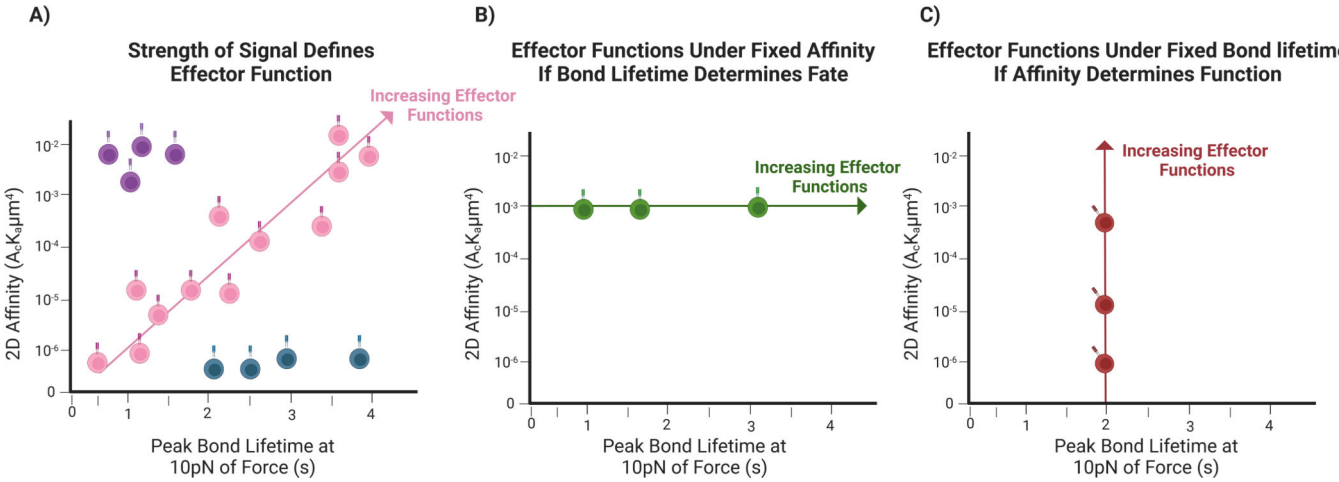

Figure 2: Force magnitude, peak bond lifetime, and affinity may be indicative of T cell effector function -

A) Schematic of correlation between peak bond lifetime at 10 pN of force and TCR effective 2D affinity. Pink line predicts increasing strength of signal and optimal effector functions as it moves to the upper right quadrant of the graph. Thus, TCRs can potentially be categorized into strong and weak agonists. This pink line indicates when bond lifetime and affinity are in lock step with one another in determining functionality. Although affinity and bond lifetime are often paired together there are instances where they do not. In these cases, it is hard to predict T cell functionality biased solely on affinity. For example, there are outlier TCRs (blue lower right quadrant) that may be highly responsive. These TCRs are low affinity but have a higher bond lifetime and may be more indicative of a TCR that is highly autoreactive. Overall, the issue with affinity prediction alone for T cell functionality is that low affinity cells are often present and highly functional with a strong strength of signal. Additional outlier TCRs, that are high affinity TCRs with a weak strength of signal and limited functionality are also commonly found. We posit that bond lifetime and ability to form catch bonds under force is a better predictor of TCR mediated responsiveness in T cells. Essentially, if there was a third axis which represents T cell function, it would more tightly correlate with bond lifetime but rather than affinity. Although affinity, bond lifetime and force magnitude are typically correlated to one another, it is possible that TCRs of similar affinity can have drastically different bond lifetime/strength projecting different T cell effector responses. For example, in **B)** the green line represents T cells with the same level of affinity but varying bond lifetimes at 10pN of force. In this case longer intact TCR:pMHC bonds correspond with increased effector function. This graph illustrates that in theory the best way to test the effects of bond lifetime under force is to decuple it from affinity, by fixing affinity. Based on current research discussed further in this review it is more likely TCR bond lifetime that predicts projected T cell effector function following activation.

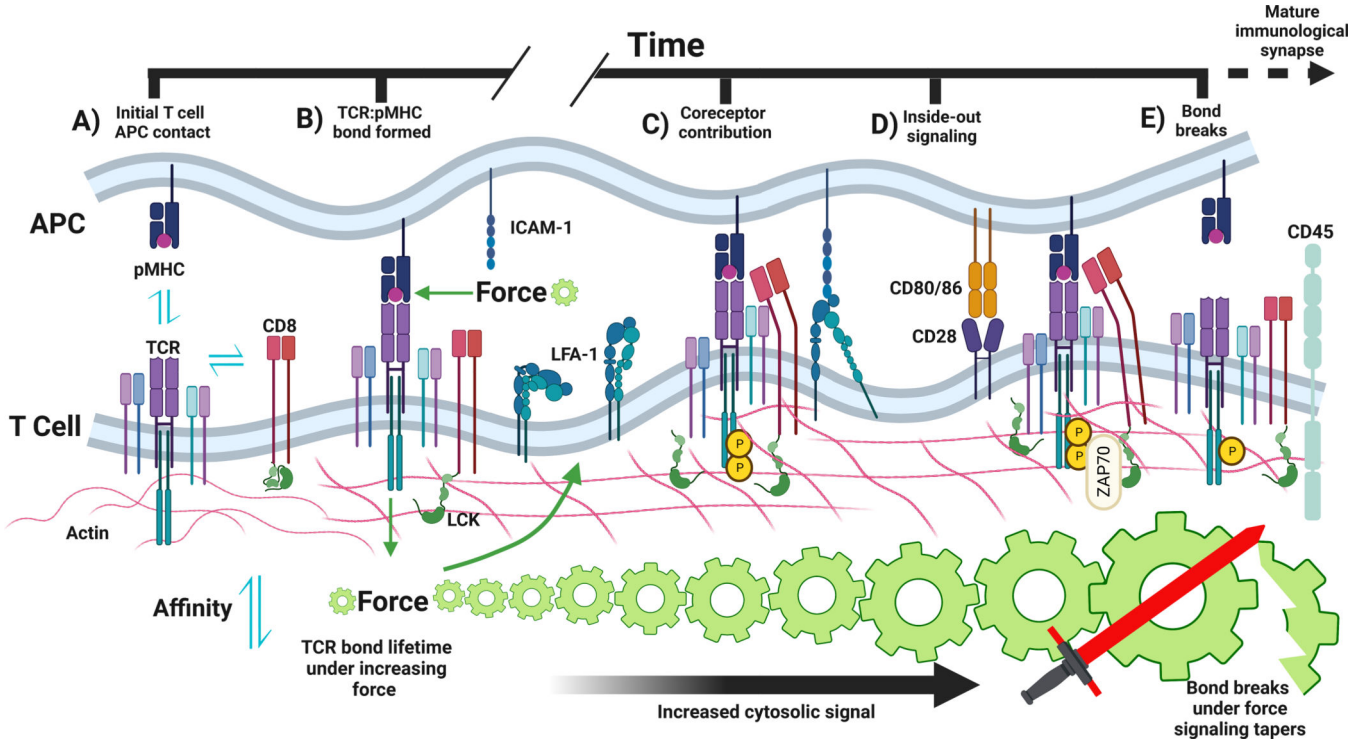

Figure 3: TCR:pMHC bond lifetime under force -

A) As an APC and a T cell approach during T cell antigen recognition pMHC and TCR are confined to the dynamic 2D environment of the plasma membrane. Affinity is what initially mediates the likelihood of a TCR:pMHC bond formation, however, after the bond is B) formed force is immediately exerted on the bond. The forces applied on the TCR:pMHC bond are in part due to the fluid motion of the plasma membrane and cytoskeleton application of inward tension. Force in this figure is denoted by green gears, increasing in magnitude for the duration the bond remains intact. C) Shortly after the TCR:pMHC bond is formed coreceptor binds MHC creating a trimolecular interaction facilitating the cytosolic phosphorylation of ITAMs by LCK. D) Prolonged bond lifetime under increased force propels a positive feedback loop that further transduces strength of signal by Zap70 and other molecules downstream of the TCR. This in turn modulates and creates a stronger TCR:pMHC bond and is known as inside-out signaling. Costimulation (CD28) and other adhesion molecules such as LFA-1 bind and further propagate the formation of the immunological synapse and full activation of the T cell although exact timing is unclear. When the synapse is fully mature larger inhibitory molecules such as CD45 are confined to the peripheral supra intramolecular cluster. In addition, it is clear that PD-1 may interact with TCR:pMHC and augment or inhibit signaling. E) the bond lifetime comes to an end and the bond breaks either due to the amplified forces that can no longer be maintained by the TCR:pMHC bond or the bond lifetime comes to an end. The pMHC then may rebind the same TCR, bind a new TCR or be pulled off the membrane by TCR known as trogocytosis.

Table I.

Collection of identified catch bonds.

BFP – biomembrane force probe; AFM – atomic force microscopy; FC – flow chamber; OT – optical tweezers; MT – magnetic tweezers

Receptor	Ligand	Technique [Citation]
Receptor	Ligand	Technique [Citation]
Selectins	Various Ligands	AFM (50–53) FC (51, 54–58) BFP (52, 56, 58–60) OT (61)
Integrins	Cell Adhesion Molecules	AFM (62) BFP (63–66)
Cadherins	Cadherins	OT (67, 68) AFM (69)
Notch1	Jag1	BFP (70)
CD44	HA	AFM (71)
TCR	pMHC	BFP (5–7, 11–13) OT (4, 8–10)
preTCR	pMHC	OT (72)