Commentary

T cells exert force for pMHC pull-ups

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T cell signaling occurs when the T cell receptor (TCR) engages antigenic peptide-major histocompatibility complex (pMHC) on the surface of antigen presenting cells (APCs). Co-engagement with co-receptor CD4 or CD8 enhances T cell signaling, especially when less biologically active pMHC ligands are used. Previous studies have shown that the TCR–pMHC bond lifetime is prolonged by a ~10–15 pN force applied via agonist pMHC (1,2). The timing, magnitude, and duration of these intermittent forces determine the calcium fluxes triggered inside the T cell (1). Lateral motions of the engaged TCRs or pMHCs are often observed at the T cell–APC interface, suggesting that bonds of pMHC with TCR and/or CD4/8 are subjected to mechanical force as these bonds have to bridge the intermembrane junction under relative motions. T cell-generated, TCR-mediated traction forces have been measured using traction force microscopy (3) and elastomer pillar arrays (4). However, no direct measurement of such forces at the per pMHC basis was available until the publication of Liu *et al*. (5).

To fill this gap in the field, Liu *et al*. developed DNA-based nanoparticle tension sensors using dual-quenched fluorophore to achieve an ~100-fold fluorescence increase over conventional molecular tension sensors. The high sensitivity of the new probes overcame the technical challenges of low number and transient interactions, enabling the authors to map the spatiotemporal distribution of forces pulled on pMHCs during initial antigen recognition, which ranged from 12–19 pN. Interestingly, this range coincides with the forces at which the TCR–pMHC catch-slip bonds reach the longest lifetime (1,2) and trigger the highest level of intracellular calcium (1).

It is important to note that the tension probe signal reports the active response from the T cell that is mediated by TCR upon engaging pMHC. Bond formation *per se*, as governed by affinity and kinetics, does not produce force. Only when the T cell responds to ligand engagement of its TCR with active generation of force and exertion of such force on TCR and CD8 to pull pMHC will the DNA hairpin unfold to display fluorescence. Liu *et al*. observed that T cells pulled on pMHCs within 40 seconds of cell–surface contact, ~40 seconds before the rise of intracellular calcium. The number of pMHCs pulled by forces beyond the DNA hairpin opening threshold increased until a steady state was reached minutes after the intracellular calcium began to decrease from its peak. These observations suggest that the generation of TCR forces is an early event preceding Ca^{2+} flux, but downstream signaling further amplifies TCR forces.

To relate the mechanical process of pMHC pulling to T cell signaling, the authors show that the TCR-induced force on surfaces coated with only pMHC requires the binding of CD8 and signaling of its associated lymphocyte-specific kinases. Additional coating of intercellular adhesion molecule 1 (which binds β2 integrins) increases the pulling force on pMHC. These results suggest that T cells harness mechanical forces to regulate antigen recognition and signal transduction.

Actin retrograde flow has been hypothesized to exert pulling and pushing forces on cell surface receptors linked to actin cytoskeleton (6). To reveal the origin of observed forces through the TCR, Liu *et al*. combined their force probe technique with fluorescent imaging of actin cytoskeleton components with high spatial and temporal resolution. Tension signals were observed to co-

localize with F-actin and be surrounded by a myosin light chain kinase ring, and were perturbed when the T cell was treated with a library of cytoskeletal inhibitors. These observations reaffirm the important roles of actin dynamics and actomyosin contractility in maintaining force through TCRs.

It is interesting to compare the magnitude of force the T cell exerts on the TCR with that of other cells exerting on well-studied receptors. Fibroblasts pull with ~40 pN forces on integrins and <12 pN forces on the Notch receptor (7). By comparison, Liu *et al*. quantified the magnitude of force on the TCR and CD8 to range from 12–19 pN by using the calibrated DNA-based nanoparticle tension sensors. Since force probes of intermediate forces were not used, the range is likely even smaller, revealing the sensitivity of TCR triggering to changes in mechanical inputs.

Liu *et al*. used a panel of altered peptide ligands (APLs) to relate antigen discrimination to force generation, showing a reduction in tension signals by biologically less potent ligands. More subtly, tension signal from APLs was delayed and followed by delayed intracellular calcium increase and impaired ZAP-70 phosphorylation. Furthermore, by using tension gauge tethers (TGTs) that provide an upper limit to pulling force (7), the authors found that using low-threshold TGTs (12 pN) resulted in no differences of ZAP-70 phosphorylation between pMHCs of decreasing potencies. On the other hand, using high-threshold TGTs (56 pN) amplified the differences in ZAP-70 phosphorylation between pMHCs in a way that is positively correlated with ligand potency. These observations strongly suggest that force generated by the T cell serves as an amplifier for TCR-mediated pMHC discrimination.

The work of Liu *et al*. has opened new research directions in T cell mechano immunology. Yet many questions remain in terms of understanding T cell triggering. For example, the density of agonist pMHC on the APC surface is generally many folds lower than what was presented in Liu *et al*., which was immobilized on glass surfaces. It has been estimated that only a small percentage of MHCs on APCs need to present foreign peptide (<1%) for T cell activation (8), with the remainder 99% of MHCs presenting self-peptides. It is of interest to look at how a T cell generates force when presented with a much lower density of agonist peptides. Prior studies have suggested that there could be a threshold of total pMHC density necessary for T cell spreading and signaling (9). To satisfy this criteria, a mixture of agonist and self pMHCs could be used to mimic the physiological proportion while maintaining an overall density. Furthermore, to fully explore the spatiotemporal distribution of TCR forces upon engagement of mobile ligands, pMHCs reconstituted onto supported lipid bilayers can be used to generate additional insight through enabling clustering of MHCs in conjunction with the inward movement of TCRs toward the center of the contact area, as these authors just did in their newest publication (10).

Finally, the findings in Liu *et al*. challenge the concept of kinetic segregation (KS) in TCR triggering. In the KS model, the close-contact zone between T cell and APCs maintains a distance of ~15 nm, which is the combined span of the extracellular domain of TCR and pMHC (11). This gap, however, is too small for large phosphatases, such as CD45, resulting in their exclusion to allow phosphorylation of signaling molecules to take place within the contact zone, which activates the T cell. However, the addition of DNA-based tension sensor introduces an extra length of >20 nm when fully extended, resulting in a gap distance of >40 nm in the contact zone. With a gap of this scale, phosphatases are able to enter the contact area despite their larger extracellular domain. The KS model has yet to explain the finding that T cell signaling was observed in the form of TCR force and calcium flux in the presence of a larger gap in the contact zone.

Overall, the results of Liu *et al*. provide direct evidence to T cell exerting pulling force specifically through the TCR, and these data highlight the importance of mechanical force in T cell triggering. To further our understanding, co-presentation of self pMHCs in addition to agonist pMHC would generate insight in conditions more similar to physiological ones. Furthermore, it is still unclear what happens before pulling forces are generated through the TCR. The path from initial triggering to the first intracellular signaling event has yet to be mapped out, but the work of Liu *et al*. clearly points us in a promising direction while providing an innovative tool for direct monitoring of mechanical events.

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Footnote

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