

A cell topography-based mechanism for ligand discrimination by the T cell receptor

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The T cell receptor (TCR) initiates the elimination of pathogens and tumors by T cells. To avoid damage to the host, the receptor must be capable of discriminating between wild-type and mutated self and nonself peptide ligands presented by host cells. Exactly how the TCR does this is unknown. In resting T cells, the TCR is largely unphosphorylated due to the dominance of phosphatases over the kinases expressed at the cell surface. However, when agonist peptides are presented to the TCR by major histocompatibility complex proteins expressed by antigen-presenting cells (APCs), very fast receptor triggering, i.e., TCR phosphorylation, occurs. Recent work suggests that this depends on the local exclusion of the phosphatases from regions of contact of the T cells with the APCs. Here, we developed and tested a quantitative treatment of receptor triggering reliant only on TCR dwell time in phosphatase-depleted cell contacts constrained in area by cell topography. Using the model and experimentally derived parameters, we found that ligand discrimination likely depends crucially on individual contacts being ~200 nm in radius, matching the dimensions of the surface protrusions used by T cells to interrogate their targets. The model not only correctly predicted the relative signaling potencies of known agonists and nonagonists but also achieved this in the absence of kinetic proofreading. Our work provides a simple, quantitative, and predictive molecular framework for understanding why TCR triggering is so selective and fast and reveals that, for some receptors, cell topography likely influences signaling outcomes.

T cell receptor | receptor triggering | single-molecule imaging | microvilli | dwell time

T cells play a central role in immunity. The triggering of T cell receptors (TCRs) expressed on the surfaces of all T cells, following their interaction with peptides complexed with major histocompatibility complex (pMHC) proteins on antigen-presenting cells (APCs), sets T cells on course to respond to pathogens and tumors (1). The TCR's capacity to distinguish between different pMHC is referred to as ligand discrimination, a process that crucially underpins immunological "self/nonself" recognition and T cell development (2). Ineffective ligand discrimination often leads to immune deficiency or autoimmunity (3). Despite its central role in immunity, the biophysical basis of ligand discrimination by the TCR is unclear, and understanding it is increasingly becoming a matter of considerable urgency. Engineered immune cells expressing repurposed or artificial antigen receptors comprise a powerful new class of cancer therapeutics (4, 5). The severe off-target activity and extreme toxicity observed in some instances (6–8), however, is at least partly reflective of our poor grasp of the interplay between TCR binding kinetics, ligand density, and discriminatory signaling.

In addition to being highly selective, TCR signaling is extremely sensitive and fast: binding to a single agonist pMHC is

sufficient to induce TCR signaling within seconds (9, 10). However, agonist peptides often comprise a very small fraction of all of the peptides presented as pMHC, raising the issue of how high sensitivity and discrimination are achieved simultaneously (11, 12). Several attempts have been made to explain ligand discrimination based on the TCR acting autonomously in ways analogous to G protein-coupled and growth factor receptors, with limited success. In such cases, TCR-induced signaling is assumed to rely exclusively on pMHC binding, and, in general, little consideration is given to extrinsic factors that might also influence signaling outcomes. Kinetic proofreading (KP)-based theories, which introduce multiple signaling steps to create delays that enhance signaling fidelity, succeed in explaining TCR discrimination in principle (13–15), but this comes at a cost, i.e., reduced sensitivity.

Significance

One approach to testing biological theories is to determine if they are predictive. We have developed a simple, theoretical treatment of T cell receptor (TCR) triggering that relies on just two physical principles: (i) the time TCRs spend in cell-cell contacts depleted of large tyrosine phosphatases and (ii) constraints on the size of these contacts imposed by cell topography. The theory not only distinguishes between agonistic and non-agonistic TCR ligands but predicts the relative signaling potencies of agonists with remarkable accuracy. These findings suggest that the theory captures the essential features of receptor triggering.

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TCR triggering results in the tyrosine phosphorylation of its cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs) by the kinase Lck, which unleashes a cascade of chemical reactions in the T cell, leading to transcriptional changes and T cell activation. In addition to ligand discrimination and sensitivity, a complete theory of T cell activation would have to account for a large number of related observations, such as peptide antagonism (16, 17), the synergistic signaling effects of self and nonself ligands (18), serial receptor engagement (19–21), and force-induced changes in TCR/pMHC stability (11, 22, 23), to name but a few. In addition, we have recently shown that TCR triggering is not strictly ligand dependent since it occurs when T cells form large contacts with non-ligand-presenting surfaces from which cluster of differentiation 45 (CD45) is at least partially excluded (24). Attempts have been made to generate models of T cell activation that incorporate the cell-biological underpinnings of many of these phenomena (25–27), but such models often have to rely on numerous assumptions, making it difficult to be certain of their accuracy (28). An alternative approach is to start with a simple model whose predictive ability can be tested, so that the extent to which it captures the essential features of receptor signaling can be determined.

Here, we developed and tested a quantitative treatment of TCR triggering relying on just two physical principles: (i) TCR “dwell time” in cell–cell contacts depleted of large tyrosine phosphatases and (ii) spatial constraints on contact size imposed by cell topography. The model suggested that restricting TCR engagement to small areas of contact would be essential for effective ligand discrimination, which could be achieved without KP. The model also predicted the relative potencies of well-characterized pMHC ligands with great accuracy, suggesting it captures the essential features of TCR triggering.

Results

A Signaling Theory Relying on TCR Dwell Time at Close Contacts. The notion that TCR triggering might depend only on TCR dwell time at phosphatase-depleted regions of close contact between T cells and APCs is embodied in the kinetic-segregation (KS) model of TCR triggering (29). The KS model proposes that, at such contacts, the TCR remains accessible to active kinases but is protected from phosphatases that would otherwise reverse its phosphorylation, resulting in the phosphorylated state being sufficiently long-lived for downstream signaling to be initiated. In this context, cognate pMHC binding, which can slow or even halt TCR diffusion (30, 31), is expected to promote signaling simply by increasing the TCR’s dwell time inside the close contact, increasing the probability of receptor triggering. Depletion of the phosphatases is considered to be a passive process, driven by differences in the size of CD45 versus that of signaling and adhesive molecular complexes that form at the T cell/APC contact (24, 32–34).

Based on these ideas, we built a quantitative treatment of TCR triggering (Fig. 1; full details of the model are given in *SI Appendix, Appendix I*). We assumed (i) that when a T cell and an APC interact, “close contacts” are formed that each partially exclude CD45 (Fig. 1A), (ii) that TCRs diffuse in and out of the close contacts (Fig. 1B), (iii) that while the TCR is bound to a pMHC ligand it is unable to leave a close contact (Fig. 1C), and (iv) that any TCR that remains in a close contact for longer than a minimum time t_{min} , irrespective of ligand binding, is “triggered,” i.e., a receptor ITAM is stably phosphorylated (Fig. 1B and C). We took t_{min} to be 2 s, in line with observation (10, 26, 35–39) and in agreement with estimates of the catalytic activity of Lck [~ 3 pTyr/s (40)] at the CD45/Lck ratio measured in contacts formed by T cells interacting with model surfaces (24). In this way, t_{min} creates an abrupt lower threshold for productive

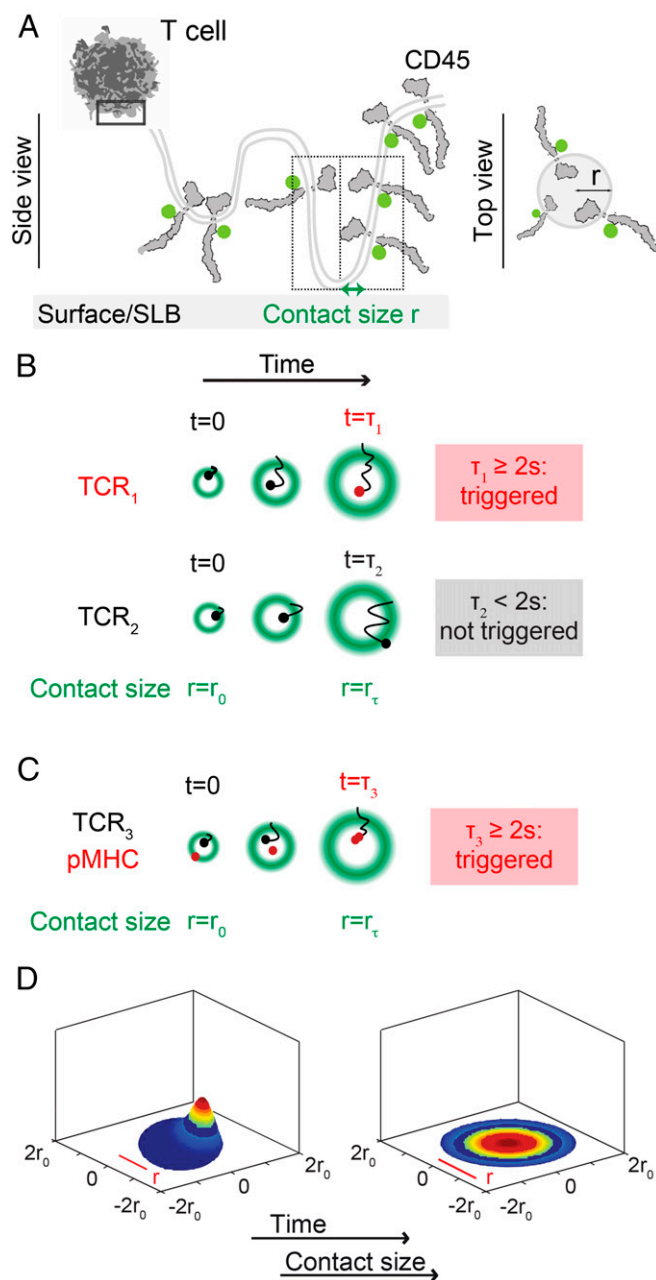


Fig. 1. A quantitative treatment of TCR triggering relying on receptor dwell time at phosphatase-depleted close contacts. (A) Top and side views of the close contact depicting contact topography (with contact radius “ r ”) and CD45 exclusion. The first box (solid line) shows the region of the cell magnified below it. The second box (dotted line) shows the region depicted in the top view on the right. (B) According to the model, a TCR (TCR_1) is triggered, i.e., phosphorylated because its residence time in the contact is ≥ 2 s. TCR_2 is not triggered because it diffuses out of the contact in less than 2 s. (C) Also according to the model, a receptor (TCR_3) that engages ligand is likely to be held in the contact ≥ 2 s and become triggered. In B and C, the margins of the contact are marked by the average positions of excluded CD45 molecules (green). (D) Snapshots from the simulation of the TCR density probability evolution in close contacts as they grow over time (*SI Appendix, Appendix I*).

residence times. In addition to t_{min} , the model incorporated the following parameters: (i) the rate of TCR entry into the close contact, (ii) the diffusion coefficients for unbound or ligand-bound receptors, and (iii) close-contact growth rate, thereby explicitly allowing for T cell topography and dynamics.

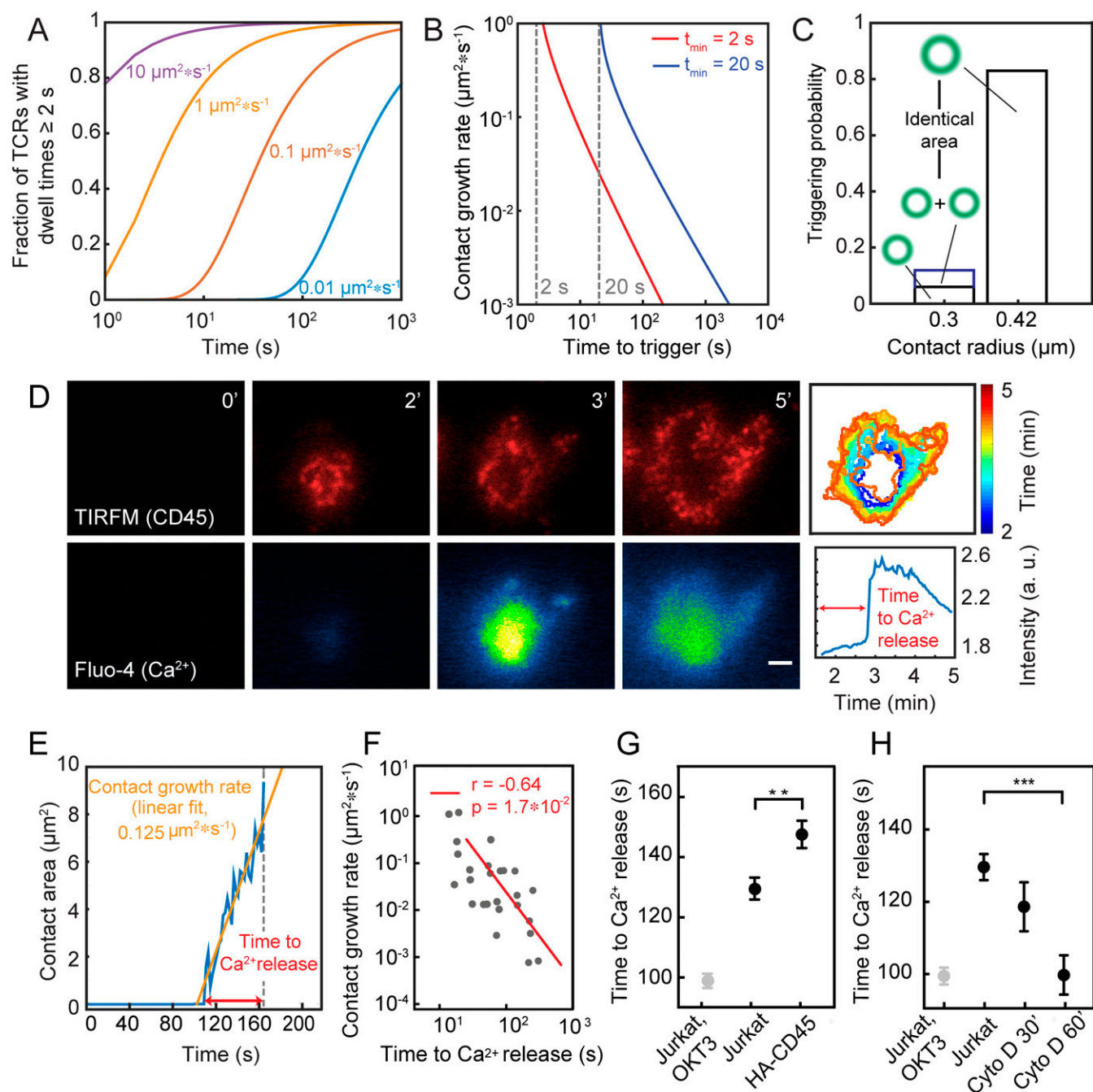


Fig. 3. Experimental validation of the model. (A) Fraction of triggered TCRs as a function of time and contact growth rate ($t_{\min} = 2$ s, $D = 0.05 \mu\text{m}^2/\text{s}$, $g = 0.01$ to $10 \mu\text{m}^2/\text{s}$). (B) Time taken to TCR triggering as a function of close-contact growth rate. (C) Comparison of triggering probability for one versus two contacts or a single contact of double the contact area. (D) Dynamics of close-contact formation [CD45 fluorescence (Gap 8.3 Fab, Alexa Fluor 568), TIRFM] (Top) and Ca^{2+} release (detected as Fluo-4 fluorescence) (Bottom) for cells contacting rCD2-presenting SLBs. (Scale bar, $2 \mu\text{m}$.) (Top Right) Color-coded representation of the temporal evolution of contact area over time. (Bottom Right) Temporal evolution of Fluo-4 intensity averaged over entire contact; $n > 10$ cells from five independent experiments. (E) Trace of a representative contact over time for growth-rate determination. (F) Relationship between close-contact growth rate and the time taken to triggering. (G) Time delay between initial contact of cells with rCD2-presenting SLBs and Ca^{2+} release for Jurkat T cells and cells expressing HA-CD45. (H) Time delay between initial contact of cells with IgG-coated glass and Ca^{2+} release in the presence of the actin depolymerizing drug cytochalasin D (data shown as mean time of calcium release for three independent experiments with >200 cells per condition; $**P = 0.01$ and $***P < 0.001$, two-tailed t test, unequal variance assumed; errors are SEM).

TCR triggering when ligands were absent ($P = 50\%$ is reached in <70 s versus 18 h; Fig. 5C).

Importantly, the model was found to robustly discriminate between ligands of different potency even at low density, a hallmark of TCR triggering. pMHC sensitivity and discrimination were found to be preserved for pMHC densities varying $>10^6$ fold, for

TCR/pMHC off rates of 1 to 50 s^{-1} , and for contact durations $t_f = 30$ and 120 s (Fig. 5D and SI Appendix, Fig. S8A). Discrimination between self and agonist pMHC was optimal for both short- and long-lived contacts between 50 and 300 nm, and lost for contacts larger than 350 nm radius (Fig. 5E and SI Appendix, Fig. S8B). Accordingly, although sensitivity was higher

radius, ligand-independent receptor triggering does not contribute toward overall triggering probability for strong agonist pMHC (k_{off} values between 1 and 10 s^{-1} ; Fig. 5F). With increasingly weaker TCR/pMHC interactions ($k_{\text{off}} = 20$ and above), the contribution of ligand-independent receptor triggering to the overall triggering probability increased but remained below 50% (Fig. 5F). For contacts with ~ 220 -nm radius, therefore, binding to pMHC is the main determinant of TCR dwell time $> t_{\text{min}}$ inside close contacts.

KP, defined by its dependence on energy-consuming intermediate steps, is often used to explain ligand discrimination by the TCR (13). In some calculations, six intermediate steps are needed to generate $>7,500$ -fold differences in the levels of TCR triggering induced by pMHC ligands differing 10-fold in affinity (13). Such large amplification mechanisms are usually only possible, however, at the expense of sensitivity (13, 15). Our calculations, which simulate a single chemical modification (TCR phosphorylation) and do not rely on a threshold for t_{min} (Fig. 5A), suggest that KP is not required for effective TCR discrimination. A 10-fold difference in affinity produced a $\sim 1,000$ -fold difference in TCR triggering for pMHC at densities of 1,000 pMHC/cell (SI Appendix, Fig. S8C), when close-contact size was restricted (SI Appendix, Fig. S8D). Even at very low pMHC densities (100 pMHC/cell), there was a ~ 100 -fold difference in TCR-triggering probability for ligands differing 10-fold in affinity (SI Appendix, Fig. S8C).

Finally, we tested whether the potency of TCR ligands could be correctly predicted, relying only on experimentally determined 2D k_{on} and k_{off} values (the parameters used are given in Table 1 and SI Appendix, Table S2). For 10 different pMHCs and a variety of ligand densities, the calculated TCR triggering probability was found to correlate extraordinarily well with signaling potency measured as IL-2 production in cocultures of peptide-pulsed APCs and T cells (Fig. 6 and SI Appendix, Fig. S11; refs. 11 and 52). The correlation was largely unaffected by the use of k_{off} values measured under force (10 pN; refs. 11, 53, and 54), which captures catch-bond behavior (SI Appendix, Fig. S12A). However, at very low ligand densities, and for pMHC with different k_{on} but similar k_{off} values, catch-bond behavior could rescue the correlation between triggering behavior and IL-2 release, which was otherwise lost (SI Appendix, Fig. S12B).

Discussion

We used a quantitative treatment of signaling to explore whether ligand discrimination and sensitivity would be achieved if TCR triggering was governed by receptor dwell time in kinase-containing, phosphatase-depleted close contacts formed when T cells interact with APCs. The model required measurements of (i) Lck activity at the levels of CD45/Lck segregation observed at the contacts, (ii) TCR density and diffusion, and (iii) the size and duration of close contacts. Validating the model in the context of ligand independent triggering, we observed that close-contact growth rate and triggering time were inversely correlated and that signaling was delayed when there was less CD45 segregation and faster when contact area was increased. Our calculations suggested that ligand discrimination and sensitivity would be possible for a triggering mechanism relying only on receptor dwell time at close contacts and that discrimination would not have to be KP-dependent. pMHC-specific responses would then be affected by the kinetics of the TCR/pMHC interaction along with TCR diffusion and T cell topography, since each of these would affect receptor dwell time.

Calculations using the model suggested that signaling outcomes in T cells would be remarkably sensitive to the size of the close contacts they formed. The probability of TCR triggering in the absence of ligands increased dramatically for close contacts with radii beyond the dimensions of contacts observed in vivo (220 nm; refs. 47, 49, 50, and 55). For close contacts like those

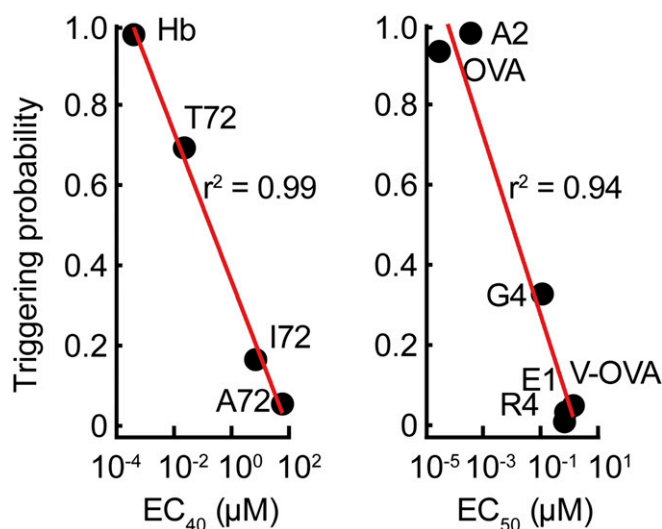


Fig. 6. Prediction of the relative signaling potencies of well-characterized TCR ligands. Peptide-stimulation potencies (EC_{40} and EC_{50} values for IL-2 secretion) for CD4^+ (Left) and CD8^+ T cells (Right) (determined elsewhere in refs. 11, 52, and 53), plotted against the probability that at least one TCR triggering event ($t_{\text{min}} \geq 2$ s) occurs at a single contact of $r_0 = 220$ nm, that persists for $t_f = 120$ s.

observed in vivo, however, a T cell would need to remain in contact with an APC for almost a day in order for a single TCR to be triggered in the absence of ligands. Thus, even though it is easily demonstrated for larger contacts in vitro (24), it seems unlikely that ligand independent TCR triggering would occur in vivo. Assuming the formation of close contacts with radii at or below 220 nm, we were able to predict the relative potency of pMHC ligands with remarkable accuracy ($r^2 = 0.94$ to 0.99). The previous best predictions were obtained by Aleksic et al. (56) ($r^2 = 0.83$), using the concept of “confinement time” (the total time a TCR is occupied by pMHC before complete dissociation). The improved predictive ability of the model likely arises partly due to our use of 2D rather than 3D binding parameters, but mostly because of the spatial constraints imposed by limiting contact size. Our analysis also showed that the level of very early signaling (i.e., ITAM phosphorylation) might be predictive of the scale of a late signaling outcome (IL-2 release). It has been suggested that forces in a cell–cell contact act to reinforce agonist binding (via catch bonds) and destabilize the binding of self pMHC (via slip bonds). We found, however, that the correlation between predicted triggering behavior and IL-2 release was largely unaffected by the use of 2D k_{off} values measured under force, except at very low ligand densities.

In contrast to most other receptors, such as G protein-coupled receptors, which are triggered in a largely binary fashion by single ligands, the TCR can react to multiple ligands varying up to 10^6 -fold in affinity (28). The discriminatory ability of the TCR has been proposed to derive from KP (13). In a previous simulation of the KS model, multiple steps producing long delays were required for effective KP because kinase activity was assumed to increase 200-fold inside versus outside close contacts, resulting in even short-lived complexes being phosphorylated (57). Our calculations suggest, however, that discrimination is achievable in the absence of KP, i.e., in a single step—TCR phosphorylation by Lck. This is possible because we assume a relatively modest increase in net kinase activity inside close contacts, based on experimental measurements in this study, which greatly reduces the likelihood that weakly bound receptors will be phosphorylated at small contacts. One interesting possibility that could be explored is that the short residence times of

self pMHC/TCR complexes and free TCRs at small contacts also make cells more sensitive to changes in dwell time resulting from agonist pMHC/TCR complex formation. The absence of any requirement for KP explains, at least in part, T cell sensitivity, with our calculations suggesting that ~200 agonist pMHC/cell would give half-maximal responses.

Our findings have several important implications. First, the size of close contacts committing T cells to synapse formation may have to be tightly controlled to avoid nonspecific activation. Defects in processes that constrain close-contact size could predispose to autoimmunity by increasing ligand-independent receptor signaling. Second, we can explain the extent to which TCR triggering is enhanced by pMHC binding, without the triggering mechanism having to be strictly ligand-dependent. For TCRs interacting with typical ligands at small contacts, we calculated that agonist-dependent signaling is favored as much as 12,000-fold over ligand-independent signaling. Third, some degree of signaling in the absence of ligand might nevertheless explain both TCR polarization and partial TCR phosphorylation (58, 59). We estimated that ~50% of TCRs remain >0.5 s inside close contacts of ~220-nm radius, yielding >1 pTyr/contact. This might not initiate downstream signaling but could generate the pMHC-independent, low-level “tonic” TCR triggering observed in vivo (58). Fourth, for close contacts increasing in radius beyond 220 nm, perhaps following an initial round of ligand-dependent signaling, ligand-independent receptor triggering might reinforce or amplify the initial response, enhancing sensitivity. Lastly, the principles established here could be extended to other ITAM-based receptors that are also sensitive to size-based changes in the kinase/CD45 ratio, such as Fc receptors (60–62), or used to calculate the binding “sweet spot” for engineered TCRs (4) or receptor mimics (61).

In conclusion, our work suggests that, rather than KP, topographically constrained T cell contact formation allows, and may even be essential for, ligand discrimination by T cells. The model’s ability to predict the relative signaling potencies of known agonists and nonagonists suggests that it captures the essential features of the TCR triggering mechanism. However, how do T cells ensure that contact size is constrained? So-called T cell “microvilli” are the obvious candidates for achieving this, although further experiments will be required to confirm whether this is true or not. Microvillus-based contacts have radii of 220 ± 20 nm (50) and persist for 1 to 5 min (55, 63, 64). Individual microvillar contacts last >6 s in the absence of cognate antigen, enough time for efficient discriminatory signaling according to our calculations. T cells may thus interrogate their targets using microvilli to exploit their unique topographic properties. Most importantly, our treatment of TCR triggering provides a predictive framework for understanding why it is selective, fast, and sensitive.

Materials and Methods

A detailed description of the mathematical model of TCR triggering and the experimental procedures for single-particle tracking, superresolution imaging of CD45, quantification of calcium release and close-contact growth, and additional control experiments are provided in [SI Appendix](#).

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