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For T Cell Receptors, Some Breakups Might Not Last Forever

Christopher C. Govern[1] and Arup K. Chakraborty[1,2,3,4,*]
[1]Department of Chemical Engineering
[2]Department of Chemistry
[3]Department of Biological Engineering
Massachusetts Institute of Technology, 770 Massachusetts Avenue, Cambridge, MA 02139, USA
[4]Ragon Institute of Massachusetts General Hospital, MIT and Harvard, Boston, MA 02129, USA
*Correspondence: arupc@mit.edu
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Does the affinity or half-life of peptide-MHC-T cell receptor (TCR) interactions determine T cell activation? In this issue of *Immunity*, Aleksic et al. (2010) propose a role for the on rate through multiple rebindings to the same TCR.

The interaction between a T cell and an antigen-presenting cell (APC) can lead to diverse outcomes ranging from survival, proliferation, and activation in the periphery to death by neglect and negative selection in the thymus. These outcomes are determined primarily, but not exclusively, by interactions between T cell receptors (TCRs) and peptide-bound MHC (pMHC). How the outcome depends on the specific features of this interaction has been greatly debated. Much research has focused on the importance of the affinity, half-life, and on rate of the bonds between TCR and pMHC, any two of which would suffice to characterize the bonds because the three quantities are simply related. Remarkably, a large amount of data over the past decade has suggested that only one of these parameters is actually relevant, although, confusingly, there has been disagreement as to which one (Stone et al., 2009). Two dominant theories suggest that T cell response correlates either with the affinity (Tian et al., 2007) or half-life of the TCR-pMHC complex (Kersh et al., 1998), with the latter gaining more acceptance. The sole dependence on the affinity between TCR and pMHC has been interpreted to mean that the T cell responds only to the equilibrium number of bonds (receptor occupancy). A dependence on half-life has been considered to be a consequence of kinetic proofreading requirements; i.e., a receptor and ligand must stay bound for a sufficiently long duration to allow a series of required biochemical changes to occur (McKeithan, 1995). In this issue, Aleksic et al. combine theory and experiment to

suggest a more nuanced view of how TCR-pMHC binding parameters influence T cell responses.

Aleksic et al. analyze a large data set on in vitro activation of a CD8^{+} T cell line bearing a transgenic T cell receptor (1G4) by cognate pMHC variants. T cell activation is assayed by measuring IFN-γ secretion in response to immobilized pMHC and by measuring lysis of peptide-pulsed APCs. They examine the relationship between activation and kinetic measurements of the rate parameters characterizing the corresponding receptor-ligand interactions. The data are particularly suited to teasing apart the roles of the affinity and the binding kinetics because they avoid some experimental difficulties that have frustrated interpretations of data in the past. For example, they eliminate pMHC stability as a confounding variable. Moreover, the kinetic measurements are carried out at physiological temperatures. Importantly, their data set contains subsets in which the on rate varies, so that changes in affinity and changes in the half-life are distinguishable.

Analyzing this data, Aleksic et al. conclude that affinity or half-life alone are not sufficient to fully explain the stimulatory potency of pMHC with varying on rates, half-lives, and affinities. The authors propose that, when on rates are fast, upon dissociation from a pMHC molecule, TCRs are likely to rapidly rebind to the same pMHC rather than diffuse away (Figure 1). Thus, they define a "confinement time," which is a measure of the total time an individual pMHC and TCR spend together regardless of interruptions between rebindings. Subsets of their data

show that T cell activation correlates well with the confinement time.

The confinement time, and thus by extension stimulatory potency, depends upon both the on rate and the half-life. Aleksic et al. show that, when the on rate is very fast or very slow, the mathematical description of the confinement time model reduces to one where activation is determined by either affinity or half-life, respectively. This suggestion, at least qualitatively, reconciles past debates on the importance of half-life or affinity of TCR-pMHC binding for stimulatory potency. The confinement time model suggests that the importance of affinity really reflects the contribution of fast on rates in increasing the confinement time. Conversely, if experiments with systems characterized by slow on rates were to exhibit a dependence of T cell activation on affinity, this would indicate a role for receptor occupancy, but such experiments are yet to be done.

The importance of TCR-pMHC rebinding for T cell activation raises the issue of the role of coreceptors given that they may increase rebinding propensity. Because CD8 binds more strongly to class I MHC molecules than CD4 does to class II MHC (Gao et al., 2002), one may ask whether TCR-pMHC rebinding plays a role in determining stimulatory potency for T helper cells. In independent work (with the Huseby lab), we have found that rebinding is important for T helper cell activation when on rates are fast and found similar correlations between stimulatory potency and kinetic parameters characterizing receptor-ligand interactions.
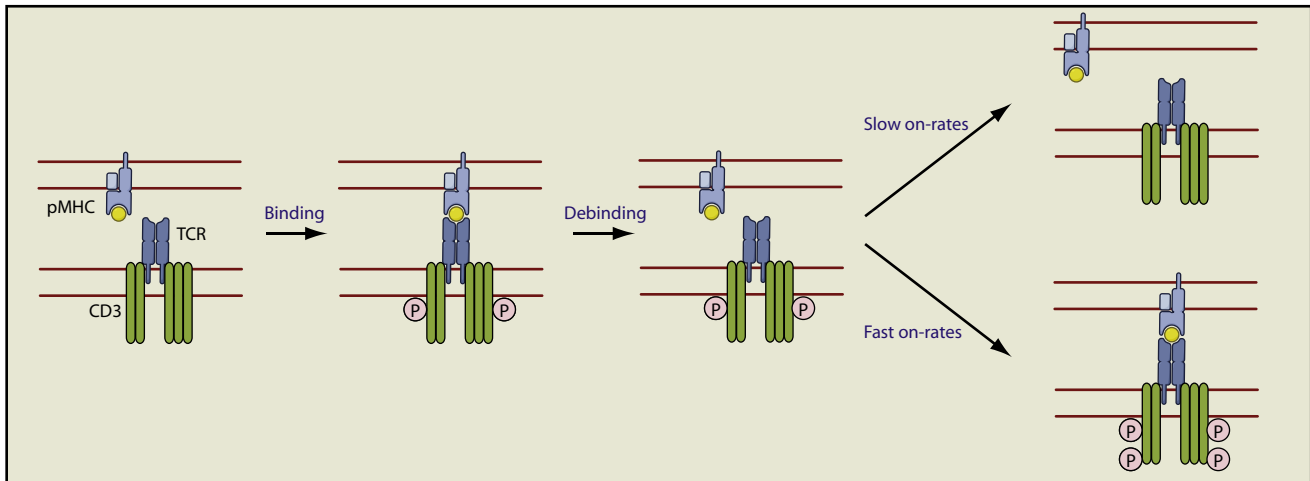
Figure 1. The Confinement Time Accounts for the Multiple Rebindings that May Occur between pMHC and TCR with Fast On Rates
In the traditional view, a pMHC and TCR bind and upon debinding, the TCR and pMHC drift apart and any signaling events are reversed. When on rates are fast, however, the TCR and pMHC may rebind before reversion of signaling events that were initiated, effectively extending the duration of the bond.

Further experimental and theoretical work is necessary to carefully examine the importance of the concept of confinement time and the functional form of how confinement time influences T cell activation. Aleksic et al. have assumed that the response of the cellular signaling machinery, measured by EC_{50}, is linear with respect to the reciprocal of the confinement time (i.e., they have shown the response is more linear with respect to this parameter than it is with respect to the affinity or off rate). Other researchers have been able to fit T cell activation potency to exponential, saturation, or even nonmonotonic curves (Holler and Kranz, 2003; Kalgeris et al., 2001; Krogsgaard et al., 2003). For ruling out affinity or half-life as explanatory models without knowing the actual form of this relationship, the best fit of the confinement time model to all reasonable functional forms (linear, saturating, etc.) must be proven to be better than the best fit to competing models. This is not a practical undertaking. Perhaps, an avenue for future research is to determine the nature of the unknown functional dependence of T cell response to receptor-ligand binding parameters. This might be accomplished by combining experimental and theoretical work on molecular events occurring on the membrane (which determine features of the peptide the T cell senses) with analyses of the signaling machinery that determines cellular response.

A more direct way to examine the importance of confinement time in physi-ologically relevant settings is to employ FRET imaging experiments in the cell-cell junction to determine whether on rates for membrane-bound TCR and pMHC are fast enough to allow frequent rebinding. What parameter ranges measured with SPR allow many rebinding events at the cell-cell junction? Recent work suggests that not just diffusion but also cytoskeleton-driven membrane motion drives TCR and pMHC apart (M.M. Davis, personal communication), and how this would influence the propensity for rebinding to the same pMHC is unknown—a careful comparison of time scales associated with receptor rebinding and local membrane motion is required. It will be important also to assess whether any peptide epitopes presented by natural infectious pathogens exhibit the high on rates required for rebinding to be important.

A key assumption underlying the confinement time model is that in the short time between rebinding events, signaling events (and signaling complexes) that were initiated are not reversed. Again, combining imaging and FRET experiments may enable testing the veracity of this assumption.

Aleksic et al. reveal the importance of rebinding for T cell activation by examining systems in which the on rate varies substantially and includes systems with fast on rates. Even more diverse data sets are required to reveal new effects and assess the importance of past proposals. For example, because they use a relatively stiff receptor, the importance of conformational flexibility cannot be assessed. Examination of diverse systems will reveal new parameters "measured" by TCR-pMHC interactions that reflect the many complexities of the 2D environment, membrane motion, molecular clustering, and the signaling network. Learning about what the T cell senses will enhance our understanding of the factors that control T cell activation and development, as well as inform the design of immunogenic peptides for vaccination protocols.

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