Lighting up TCR takes advantage of serial triggering

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The complex interaction with APCs that is required for T cell activation is not well understood. A combination of experimental data and mathematical modeling provides insight into the competition between serial triggering and kinetic proofreading.

Recent years have witnessed great progress in our understanding of T cell recognition of antigen-presenting cells (APCs) and the subsequent activation that leads to effector functions. This is due to some important discoveries, including vivid observations of the redistribution of cell-surface molecules to form the immunological synapse[1,2], advances in our understanding of the signaling cascades initiated by recognition of APCs[3,4] and observations of the spatio-temporal evolution of intracellular signaling molecules during activation[5–7]. Despite these advances, a clear picture of the factors that determine T cell activation and commitment to proliferation or those that determine selection of immature T cells in the thymus is missing. For example, we do not have a proper understanding of the ways in which extracellular receptor–ligand binding and intracellular signaling cascades are synchronized[5] or how the immunological synapse regulates these events. The proliferation of research exploring various facets of these problems, however, promises much progress in the ensuing years. In this issue of *Nature Immunology*, Coombs *et al*. provide an insight that is expected to be important for future studies[8].

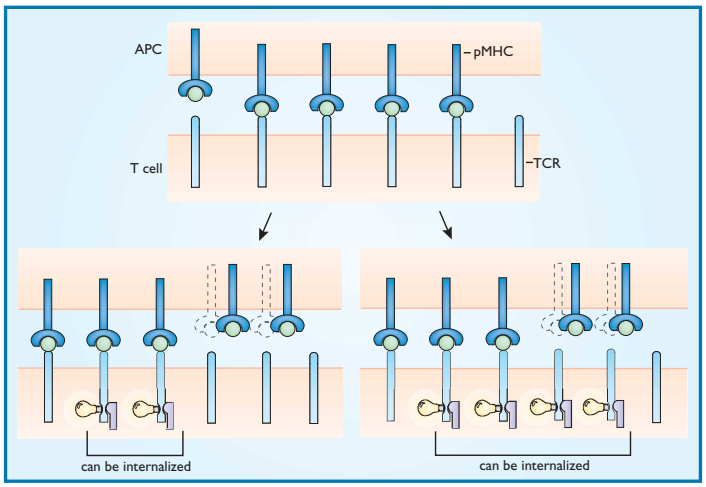

Figure 1. "Lighting up" of TCRs for internalization. The marking of TCRs for internalization can be appreciated by considering a movie of the dynamic interaction between pMHC on an APC and TCRs on a T cell (upper panel). Snapshots from this movie show two possible scenarios. TCRs are subjected to internalization ("lit-up") if they have been bound to pMHC for sufficient time to complete biochemical changes for triggering and (**a**) they are still bound to the pMHC (model 1) or (**b**) they are either still bound or have dissociated from the pMHC (model 3).

T cell activation is initiated by the binding of T cell receptor (TCR) molecules on its surface to appropriate peptide–major histocompatibility complex (pMHC) expressed on the surface of APC. T cells are highly sensitive and selective in that they can be activated by APCs bearing just a few pathogen-derived pMHC molecules in a sea of self-pMHC. A clear understanding of how T cells accomplish this Herculean task remains elusive.

Proposals that partially resolve these issues have been suggested. The hypothesis of serial triggering was introduced to explain how a few agonist pMHC molecules could activate many TCR molecules[9,10]. The basic idea is that a single agonist pMHC sequentially binds to many TCRs, thereby triggering them in a serial fashion. Serial triggering, by itself, implies that shorter half-lives for bound pMHC-TCR would be more efficient for activating T cells. However, the concept of kinetic proofreading set limits on how short the half-life can be in order for T cell activation to occur[11]. This model proposes that a series of biochemical steps—which require a certain time, τ—must be completed after pMHC-TCR binding in order for the TCR to be triggered. Thus, the half-life of bound pMHC-TCR must be sufficiently long to enable TCR triggering. The competing requirements for enhanced serial triggering and kinetic proofreading suggest an optimal half-life for pMHC-TCR binding. Indeed, for some time, data on the dependence of T cell activation on pMHC-TCR binding kinetics has suggested this[10].

One consequence of TCR triggering is that these molecules undergo endocytosis. Coombs *et al.* present experimental data to demonstrate that TCR endocytosis is maximized for a particular value of the half-life of bound pMHC-TCR[8]. They analyze this data using a mathematical model and show how T cells take advantage of serial triggering. The mathematical model uses the language of partial differential equations to describe the following processes: pMHC-TCR binding and dissociation, characterized by an on rate and an off rate; TCR internalization after it has completed the requisite biochemical steps, characterized by a rate of completion of these biochemical steps and a rate of TCR internalization; and diffusion of receptors and ligands in the contact area, characterized by diffusion coefficients. Allowing for binding, dissociation, diffusion and rebinding implies that pMHC can naturally bind serially to many TCRs. Only an infinite half-life for pMHC-TCR or a value of 0 for all diffusion coefficients (that is, immobilized receptors and ligands) prohibit serial binding in this model.

Coombs *et al.* examined three models for TCR endocytosis[8]. TCRs are candidates for internalization if they have been bound long enough to complete the biochemical steps necessary for triggering and are either (model 1) still bound to pMHC or (model 2) have since dissociated from pMHC. Ostensibly, these two models may occur simultaneously in the same setting, which gives rise to model 3. They find that only the latter two models are consistent with an optimal half-life for TCR internalization.

This result can be understood in simple terms as follows. Imagine watching a movie showing pMHC and TCR molecules binding, dissociating and diffusing. If at any time, a TCR molecule becomes a candidate for endocytosis, then it lights up (**Fig. 1**). We stop the movie after a number of frames corresponding to a time longer

than that required for triggering (τ) and ask how may TCRs are lit up. Some fraction (determined by the rate of endocytosis) of these candidates for endocytosis are then internalized. The movie is then restarted, and the process is repeated.

The three models considered by Coombs *et al.* correspond to different rules for determining candidates for endocytosis[8]. In the first model, each time the movie is stopped, the only TCRs that are lit up are those that are still bound to pMHC and have been bound for at least a time equal to τ (**Fig. 1a**). The fraction of TCR molecules that are lit up is thus simply the probability of finding pMHC-TCR complexes that stay bound for longer than τ. Clearly, the probability of finding candidates for endocytosis monotonically increases with the half-life of the pMHC-TCR bond. The monotonic increase in the pool of candidates for endocytosis with an increasing pMHC-TCR half-life also results in a steady increase in the number of internalized TCRs. Thus, by solving their mathematical equations, Coombs *et al.* find that this model is inconsistent with the experimental observation of an optimal half-life for maximal TCR endocytosis[8].

Models 2 and 3 of Coombs *et al.* lead to similar results, and we can understand the behavior of both models by considering one of them. In model 3, each time the movie is stopped, two types of TCRs are lit up: TCRs that remain bound to pMHC for longer than τ and those that are now free but were bound to pMHC longer than τ before dissociation. (The authors put an additional constraint on the latter by insisting that these TCRs are no longer lit up after a certain time has elapsed. They estimate this time period is many minutes. This constraint does not change the qualitative argument). As snapshots from the movie show, if the half-life of the pMHC-TCR bond is sufficiently long, this scenario leads to an increased pool (compared to model 1) of candidates for TCR endocytosis (**Fig. 1b**). In addition to TCRs that are lit up according to the rules of model 1, other TCRs that are now free—but have been bound long enough—are also lit up. This model is true only if the half-life is sufficiently long. Otherwise, previously bound TCRs would have a small chance of having been bound long enough to light up. Thus, we must consider two regimes for models 2 and 3. When the half-life is sufficiently long, most TCRs that have been bound previously were bound for longer than τ, and there is a marked increase in the pool of candidates for endocytosis when TCRs remain lit up after dissociation. The number of available candidates (and amount of TCR endocytosis) increases as the half-life becomes shorter because this allows pMHC molecules to light up a larger number of TCRs. This is the regime in which the effects of serial engagement are dominant. On the other hand, short half-lives correspond to the regime in which the effects of kinetic proofreading are dominant and TCR endocytosis decreases as half-life becomes shorter. In this regime, TCR endocytosis depends on the half-life in a manner that is opposite to the case in which serial triggering is dominant. Maximizing the number of unbound, but lit up, TCRs requires balancing these two trends. This results in the peak for TCR endocytosis as a function of half-life reported by Coombs *et al.* [8].

The above narrative of the movies provides simple explanations for the finding that the TCR must stay lit up after dissociation in order for T cells to take advantage of serial triggering and show a nonmonotonic dependence of TCR endocytosis on pMHC-TCR half-life. The biochemical change that marks a TCR molecule for internalization is an issue that must be resolved by future experiments. How is this biochemical modification preserved after dissociation? Could it be preserved by binding to self-pMHC?

Insight into appropriate models for TCR endocytosis was obtained by Coombs *et al.* by the juxtaposition of experimental data and the predictions of a mathematical model[8]. Mathematical models can assist in the search for mechanistic insight by quantitatively evaluating the effects of competing forces in different conceptual models and eliminating those that are inconsistent with experimental data. One expects that synergistic use of mathematical models and experimentation will be more common as we continue to search for mechanistic insights into T cell activation and selection in the thymus.

Coombs *et al.* have studied a model that considers only pMHC-TCR binding; the consequences for signaling are subsumed in composite parameters. In addition, they do not consider synapse formation or the role it plays in regulating intracellular signaling cascades. A model that marries mechanisms for synapse formation[12] with a detailed signaling model[3,4] will be useful in the quest for further mechanistic insight into the mysteries that underlie the enormous sensitivity of T cells in orchestrating an immune response. The insight provided by Coombs *et al.* into how TCRs remain marked for internalization provides important input to the development of such models.

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Two faces of caspase-8

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A deficit of caspase-8 should presumably lead to over-activation of lymphocytes. A recent report in *Nature* from Lenardo's group, however, describes humans with a severe caspase-8 deficiency whose T cells, counter-intuitively, have impaired activation abilities.

When the first caspase was discovered 10 years ago, it was named ICE (interleukin-1β converting enzyme) for its ability to process cytokines, specifically interleukin 1 (IL-1)[1,2]. In the years following this discovery, an enormous body of work has been generated that describes the characteristics of caspases, and ten additional caspases have been identified in humans. However, this work has revealed that most caspases function in