can be easily instrumented. These zones, distinct from the classic subduction-zone settings that account for most of the Earth's seismicity, need to be intensely monitored over a broad area. These data also point to the importance of faults on all scales (not just the largest) as promising locations for detecting precursors. Hydrologic phenomena naturally occur in faulted regions where water often flows readily. Hydrothermal areas denote relatively deep-seated faulting and are therefore of particular interest (*19*). Faults are also regions that by definition concentrate strain and thus should amplify coseismic and precursory deformation. Hydrogeochemical indicators may thus simply be in the right location (*20*). In several cases, borehole strainmeters have detected many of the precursors seen by hydrogeochemical phenomena. This provides a critical link with strain measurement and suggests that such strainmeters are particularly well suited for detecting precursors. In many ways, the onland San Andreas fault system, embedded within a relatively simple, well-studied, 1000 km by 200 km plate boundary zone, is ideal for the study of precursors. At present, however, this zone is only sparsely instrumented (in terms of hydrogeochemical and related strain instrumentation), especially when compared with seismogenic zones in Japan and China. In addition, sites have been concentrated primarily along one section of the San Andreas fault in association with the Parkfield Experiment.

For many researchers at the meeting, there was a sense of déjà vu, as if the Izu-Oshima and successfully predicted Haicheng earthquakes had recurred two decades later. The earlier events led to great excitement in the seismological community and a feeling that earthquake prediction was finally within our grasp. Yet, this goal has been elusive, gradually leading to strong skepticism, especially in the United States, about the prospects for prediction and even for detecting precursors. But this present pessimism, like the initial optimism, is probably excessive. We now realize that the earthquake prediction problem is not easy. Solving it requires a sound physical model and sound observations; these observations require patience as we wait for earthquakes to occur. Although we cannot avoid this waiting process, it can be accelerated considerably by casting a wider net—expanding observing programs to capture many more events and their precursors—and by fostering the kind of international cooperation seen at this meeting.

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How T Cells Count

Ellen V. Rothenberg

The surfaces of the antigen-presenting cells of the body offer a bazaar of foreign peptides, bound to their major histocompatibility complex (MHC) molecules, for the sampling and possible activation of the T cell population. To mount an immunological defense, T cells must recognize these foreign peptides. The T cell receptor (TCR) interacts with the foreign peptide–MHC complex, sometimes activating the T cell. How does the T cell know when the interaction should result in activation? In a report in this week's issue, Viola *et al.* (*1*) show that T cells "count" the number of TCRs engaged by the peptide-MHC complex and become activated when that number reaches about 8000.

In practice, TCR-peptide-MHC recognition is very sensitive. T cells, each with more than 10,000 TCRs that bind to a specific antigen, can respond if they recognize the peptide associated with as few as ~100 of the 100,000-odd MHC molecules on an antigen-presenting cell (*2–4*). Yet the interactions of TCRs with their ligands are weak, with dissociation constants of 10^{-6} M or less, suggesting that a TCR is engaged by its antigen for only a short time. In spite of this weak interaction, an individual T cell can distinguish variants of the same peptide, bound to the same MHC molecule, often responding to its optimal TCR ligand but not to a slightly altered form.

In fact, all mature T cells that recognize foreign antigens have TCRs that can also interact—even more weakly—with structurally related self antigens. Such an interaction with self peptide–MHC complexes in the thymus is essential to allow developing T cells to complete their maturation (*5*). How can enough information for several, critically distinctive responses be transduced by such a small number of weak interactions?

Last year, Lanzavecchia and colleagues (*6*) provided a striking clue toward resolving this problem. They found that each peptide-MHC complex did not bind stably to one complementary TCR complex but could engage multiple TCRs serially, ~100 to 200 TCRs per peptide-MHC complex. Because more TCRs are removed from the cell surface when there is more stimulating ligand, a lower limit for the number of binding events could be calculated by measuring the number of TCRs lost. These results indicated that TCR-ligand interactions are fundamentally dynamic and led to explanations of several features of T cell activation. First, they suggested that the moderately low affinity of binding might be an asset rather than a liability for T cell activation, enhancing the efficiency of rebinding by a small number of ligands. Second, they suggested that T cells might use multiple rounds of binding and dissociation to amplify small affinity differences for discrimination of optimal and altered peptide ligands. This mechanism would be particularly useful if T cells use "kinetic proofreading" to tell whether an optimal or a variant peptide ligand is binding (*7, 8*). Finally, they suggested a way to reconcile the brief encounters of individual TCR-ligand binding events with the requirement of T cells for several hours of stimulation before committing to an activation response (*9, 10*). All these predictions, however, depended on resolving a critical question: whether the removal (or "consumption") of so many

The author is in the Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125, USA. E-mail: evroth@cco.caltech.edu

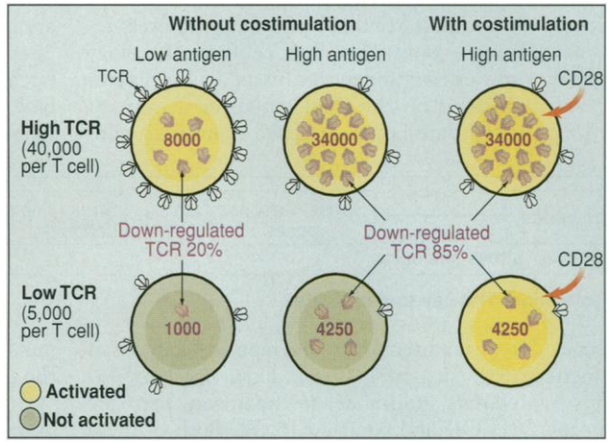
TCRs was important as an activation signal for T cells or was simply a byproduct of inefficient activation by low-affinity ligands.

The work by Viola *et al.* (*1*) shows that the consumption is a key signal. T cells commit themselves to activation only when a certain threshold number of TCR complexes have been engaged and down-regulated. This threshold is surprisingly consistent for different TCR ligands—whether an antigenic peptide, a superantigen, or an antibody to the TCR. This constancy is especially striking because the dose-response curves for T cell responses to these ligands reach the threshold at different doses and show different dose-response slopes and plateaus, reflecting the different affinities of binding and different geometries of interaction. Peptide-MHC complexes do not "consume" more TCR complexes than do immobilized monovalent antibodies to CD3 before reaching the threshold; they reach the threshold after engaging the same number of TCR complexes. Low-affinity ligands actually reach the threshold with less ligand than does the higher affinity antibody, which cannot engage serially.

The key parameter is the absolute number of TCRs engaged, which is surprisingly high (~8000 per cell). As a direct consequence, T cell responsiveness is disproportionately sensitive to the absolute number of TCRs on the cell surface, not just to the TCR-ligand binding affinity (see figure). The affinity controls the percentage of a T cell's receptors engaged as a function of ligand dose (by controlling the probability that any given TCR will be engaged), but even a high-affinity interaction and a dose of ligand yielding nearly 100% engagement can fail to activate if the absolute number of TCRs involved is still below 8000. Cells that naturally have low TCR numbers, or cells with previously down-regulated TCRs, may require unattainably high concentrations of antigen to engage enough receptors for (re)stimulation. Indeed, cells that escape the thymus with potentially autoreactive TCRs often have low TCR surface densities (*11*). The results of Viola *et al.* confirm that this mechanism should be an effective way, without killing these cells, of excluding them from reactions with any ligands except unusually avid ones.

The TCR threshold is "tunable" in two distinct ways. First, preactivated and resting cells differ in the amount of antigen needed to down-regulate the same number of TCRs. The actual threshold number for activation remains the same, but in the resting cells, almost 100 times as much MHC-peptide or MHC-superantigen complex is needed to engage the threshold number of TCRs as in the preactivated cells. Notably, this makes the "low-affinity" ligand as inefficient in TCR engagement as is the high-affinity antibody to CD3, which is not altered in its dose-response curve.

Second, the threshold number is reduced by simultaneous stimulation of another cell surface protein, CD28, in both resting and preactivated cells, collapsing from about 8000 to 1500 or less. In addition to overcoming a shortage of engaged TCRs, CD28 costimulation may assist in driving a response toward activation rather than anergy by other mechanisms. Thus, T cell triggering is controlled by (i) local antigen concentration, (ii) the threshold number of TCRs as a fraction of a cell's total TCRs, (iii) the ability of the cell to engage its TCR sequentially, and (iv) the availability of costimulation (see figure).



Counting to 8000 before activation. Effects of various amounts of TCR, ligand, and costimulation by CD28 on T cell activation. For simplicity, the down-regulated TCRs are portrayed as internalized.

How does the T cell "count" the number of TCRs engaged? Those cells with an insufficient number of engaged TCRs undergo a substantial but abortive Ca^{2+} flux, in sharp contrast to the sustained Ca^{2+} increase in cells that have reached threshold. This observation crucially links the cumulative number of TCRs down-regulated to the overall duration of signaling. Exactly how this process occurs is a fascinating question; one particularly interesting possibility would be that the internalization delivers the TCR-associated kinases and other signaling molecules to new intracellular compartments before they stop signaling, for interaction with new substrates. In this instance, the cell could distinguish between signals from tightly binding, stable ligands and weakly binding, serially engaged ligands by the ratio between products of the membrane-tethered and the hypothetical internalized complexes. Perhaps a distinction between stable and serial triggering might underlie the choice between negative and positive selection in developing thymocytes.

A quantitative triggering threshold, such as Viola *et al.* have described, is likely to be a vital component of the mechanism T cells use to measure "danger." Matzinger has recently argued (*12*) that the immune system must supplement basic self-nonself discrimination with a metric that assesses local "danger," and responds forcefully only in instances where there is tissue damage or another sign of serious threat. Such a mechanism is needed because T cell recognition, in fact, is quite flexible (*5*, *13*). Only a few amino acid side chains of an antigenic peptide engage the TCR directly, and it is likely that many foreign antigens could resemble at least some endogenous peptide. Thus, the T cell repertoire cannot afford to be completely purged of reactivity with all self antigens. The compromise is to delete T cells with high-affinity receptors for self antigen and receptors for antigens that are common on "professional" antigen-presenting cells, that is, hematopoietic cells specialized to provide optimal costimulation. This tolerogenic mechanism simply needs to be set to keep any interaction of TCRs with antigens on nonprofessional antigen-presenting cells below the threshold (*11*, *14*), reserving those TCRs for responses to foreign antigens in instances where there is danger. Induction of costimulatory molecules, which can collapse the TCR engagement threshold to a low number, can be one outcome of inflammation and tissue damage in a dangerous environment. Now, also, we must consider that the sudden increase in concentration of a peptide from its physiological steady state to a threshold-breaking level may be a basic, primitive warning of the presence of a replicating pathogen.

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