Understanding the Mechanisms of Sustained Signaling and T Cell Activation

By Antonio Lanzavecchia

From the Basel Institute for Immunology, CH 4005 Basel, Switzerland

A Dynamic View of T Cell Behavior. For many years it has been known that T lymphocytes engage APCs in a long-lasting interaction that results in the generation of a rapid and sustained signal in T cells. Lasting almost 1 h, this sustained signal is required by T cells to maintain the transcription factors translocated in the nucleus and, ultimately, to become activated (1). However, in view of the low number of ligands recognized and of the low affinity of TCR, the question of how T cells maintain the sustained signal and become activated remained a puzzle (2).

Unlike the antigen–antibody interaction that can be easily studied by measuring the proportions of the reactants at equilibrium, the interaction of the TCR with its ligand has proved to be much more difficult to analyze. This interaction occurs in the limited space between the opposing membranes of two motile cells, the T cell carrying $\sim$30,000 TCRs and the APC displaying few ligands, typically $\sim$100 complexes for recognition by T helper cells (3, 4) and perhaps as few as one complex for recognition by cytotoxic T cells (5).

Because of the small number of ligands recognized, it was originally thought that only few TCRs would be engaged and would be sufficient to initiate and sustain the signal transduction cascade for the time necessary to lead to T cell activation. In other words, it was thought that, once the appropriate molecular interactions had been set in place, the signal would be self sustained. In the last three years a kinetic and stoichiometric analysis of the TCR-peptide–MHC interaction has revealed a very dynamic type of interaction.

Two experimental approaches have proved particularly useful for studying the dynamics of TCR-peptide–MHC interaction in living cells. The first is the continuous monitoring of the TCR-mediated signaling that can be achieved by measuring intracellular calcium elevations or medium acidification. These methods have been used to demonstrate that the sustained signal in T cell–APC conjugates requires a continuous engagement of TCRs with peptide–MHC complexes on APC. Indeed, treatments that prevent these continuous engagements such as addition of anti-MHC antibodies, blocking of the actin cytoskeleton, or removal of peptide antigen, can terminate the signaling process within 2–4 min, demonstrating that the signal emanating from triggered TCR is short lived (6, 7).

The second approach is the measurement of the stoichiometry of the TCR–ligand interaction. Valitutti et al. have shown that TCR downregulation is a reliable readout of the number of TCRs triggered. Using this method it was shown that, over time, as many as 20,000 TCRs are triggered by as few as 100 peptide–MHC complexes (8). Thus the fast kinetics of TCR–ligand interaction appears to be a key feature allowing multiple TCR engagements by a single agonist (for review see reference 9). In addition, T cells appear to be able to take a "count" of the number of TCRs triggered and respond by proliferation and cytokine production when an activation threshold is reached (10).

Three papers in *The Journal of Experimental Medicine* have used these methods to reveal new aspects of TCR signaling induced by peptide–MHC complexes with agonistic or antagonistic properties and by APC with different costimulatory capacities.

Sustained Signaling Inhibited by TCR Antagonists. The paper by Wülfing et al. (11) analyzes the calcium signal at single cell level in T cells activated by a variety of ligands alone or in combination. The authors distinguish four patterns of intracellular calcium elevation. Although agonist induced a robust response that correlated with T cell proliferation, three antagonist complexes gave very different release patterns, or none at all. In addition, when added together with the agonist, the antagonists markedly reduced the calcium response. These results demonstrate that antagonism does not require calcium release, but can inhibit the calcium signal triggered by agonists. Interestingly, the authors report an inverse correlation between the strength of the stimulus and the time required for the onset of the calcium signal after T-APC contact. The dose dependence of this delay has been interpreted to indicate that the rate-limiting step in the calcium response involves accumulation of some form of intracellular complex or molecule with a half-life of a few minutes.

The paper by Preckel et al. (12) reports that class I–restricted CTL specific for haptenated peptides can be antagonized by ligands carrying subtle differences in either the hapten or the peptide. Interestingly, although agonists induced a dose-dependent downregulation of TCR, antagonists failed to do so, despite engaging the specific TCR. Moreover, when offered together, the antagonist inhibited TCR downregulation induced by agonists. In this system, no sign of anergy or receptor inactivation was evident. Instead, these results suggest that antagonists block T cell functions by competitively engaging the specific TCR in unproductive interactions that do not result in downregulation, nor in inactivation or anergy.

Taken together, these two papers make an important

contribution to understanding TCR antagonism (13–15). They demonstrate that antagonists not only fail to induce a calcium signal as well as TCR downregulation, but also, and more importantly, inhibit calcium signal and downregulation induced by agonists. These results are compatible with the notion that sustained signaling requires a minimum rate of TCR triggering to produce sufficient concentrations of second messengers. It appears that, either because of their higher concentration or because of their faster kinetics (16), antagonists can rapidly engage, in a nonproductive fashion, a large number of TCRs, thus leaving only a few available for triggering by agonists. In this way, the antagonists decrease the rate of TCR triggering below that sufficient for sustaining the signal. Indeed, previous work has shown that reduction in the number of TCRs has a dramatic effect on the capacity of T cells to respond to low concentrations of agonist and to sustain the signaling process (10).

TCR Occupancy and T Cell Activation. A third paper by Cai et al. (17) investigates the requirements for TCR downregulation and T cell activation in naive CD8$^+$ T cells using peptide–MHC complexes of different affinities for the TCR. The APCs used by the authors are *Drosophila* cells, engineered to express extremely high levels of a single peptide–MHC complex in the total absence or in the presence of adhesion and costimulatory molecules. Using these extreme experimental conditions, TCR downregulation can be dissociated from T cell activation. The authors show that when costimulatory APCs are used, T cell activation can occur in the absence of measurable TCR downregulation, whereas with APCs that display the agonist in the absence of adhesion and costimulatory molecules, no activation is observed in spite of an almost complete TCR downregulation. The fact that the authors do not find a correlation between the extent of TCR downregulation and T cell activation is not entirely surprising. Indeed, it is well known that the final outcome of TCR triggering (signal 1 as measured by TCR downregulation) is modulated by additional stimuli provided by costimulation (signal 2) (18). In a previous study done on human T cells, it was shown that in the absence of costimulation, a relatively large number of TCR have to be triggered (~8,000) to activate a T cell, whereas this threshold is decreased to ~1,500 if costimulation is provided (10).

The findings by Cai et al. are also important for our understanding of the nature of the ligands that induce TCR downregulation, as well as for the mechanism of downregulation itself. As to the first point, all the data reported so far indicate a good correlation between the capacity of a ligand to induce TCR downregulation, and its strength as an agonist (8, 10, 12, 17). Indeed, weak agonists have a lower capacity to downregulate TCR that exactly reflect their reduced capacity to induce calcium signal and to activate T cells to produce cytokines, while antagonists fail to do so. As to the mechanism, Cai et al. show that TCR downregulation is not inhibited by genistein or azide. Further evidence indicates that TCR downregulation is also resistant to inhibitors or depletion of protein tyrosine kinases

and results from targeting and degradation of TCRs in the lysosomes (19).

From Serial Engagements to T Cell Activation. The sustained signaling in T-APC conjugates appears to be maintained by a very dynamic mechanism (Fig. 1). The fast kinetics of interaction allows a single peptide–MHC complex to engage many TCRs over time. The fate of these engagements can vary depending on the stability of the interaction which can be influenced by the presence of a coreceptor. Engagements that are stable enough result in complete assembly of the signal transduction machinery and triggering of the TCR. This leads to the production of second messengers that accumulate to the point where a calcium signal is released. However, these messengers are short lived and, in the absence of a continuous TCR triggering, the signal will stop. This happens experimentally when we interfere with TCR–agonist interaction or spontaneously as a consequence of TCR downregulation (20). By serially engaging and triggering TCRs, few agonists can maintain sufficient levels of second messengers to sustain the signal.

How can antagonists interfere with serial triggering by agonists? Because of their fast kinetics (16), antagonists can engage TCRs very efficiently. However, although we cannot exclude the possibility of a transient inactivation of some substrates of the signal transduction cascade, it seems
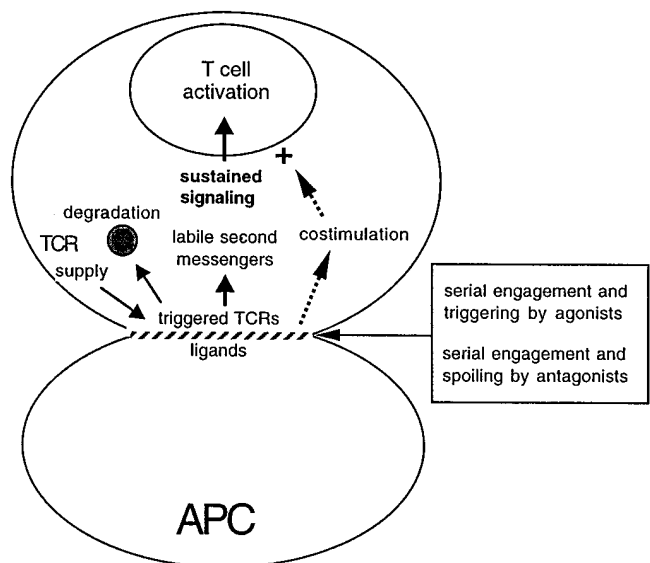
Figure 1. From serial engagements to T cell activation. In the area of contact between T and APC, few ligands serially engage TCRs. The duration of the interaction determines whether the TCR is triggered or not. Strong agonists are efficient at serially triggering TCRs, whereas antagonists fail to trigger and spoil many TCRs, thus effectively competing with agonists. Once triggered, the TCRs generate labile second messengers and signal for a short time until they are eventually degraded in lysosomes, whereas new TCRs are recruited in the contact area. When a sufficient concentration of second messengers is reached, the calcium signal is released. This signal is sustained so long as a minimum rate of TCR triggering is maintained. The sustained signaling is required for induction of transcription of cytokine genes, resulting in T cell activation. Costimulatory signals generated in the area of contact can modulate the outcome of those emanating from the TCR and effectively decrease the number of TCR-triggering events required to induce T cell activation.

that challenge with antagonists per se does not lead to any form of signal transduced, neither is there any long- or short-term consequence on T cells. The serial spoiling of TCRs by antagonists could be the basis for their capacity to effectively reduce the rate of triggering by agonists.

How do we go from serial engagement to T cell activation? The possibility of measuring signal 1 using TCR downregulation has provided a quantitative framework to the notion that the effect of the signals emanating from TCRs can be influenced by signals delivered by costimulatory molecules (18). Clearly, the level of TCR engagement required to trigger a T cell is very high in the absence, and much lower in the presence, of costimulation. Understanding the nature of the costimulatory signals and how they are integrated those from TCR will be a major task for future research.

References

1. Ulman, K.S., J.P. Northrop, C.L. Verweij, and G.R. Crabtree. 1990. Transmission of signals from T lymphocyte antigen receptor to the genes responsible for cell proliferation and immune function: the missing link. *Annu. Rev. Immunol.* 8:421–452.

2. Karjalainen, K. 1994. High sensitivity, low affinity–paradox of T-cell receptor recognition. *Curr. Opin. Immunol.* 6:9–12.

3. Harding, C.V., and E.R. Unanue. 1990. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. *Nature (Lond.).* 346:574–576.

4. Demotz, S., H.M. Grey, and A. Sette. 1990. The minimal number of class II MHC–antigen complexes needed for T cell activation. *Science (Wash. DC).* 249:1028–1030.

5. Sykulev, Y., M. Jooh, I. Vturina, T.J. Tsomides, and H.N. Eisen. 1996. Evidence that a single peptide–MHC complex on a target cell can elicit a cytolytic T cell response. *Immunity.* 4:565–571.

6. Valitutti, S., M. Dessing, K. Aktories, H. Gallati, and A. Lanzavecchia. 1995. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. *J. Exp. Med.* 181:577–584.

7. Beeson, C., J. Rabinowitz, K. Tate, I. Gutgemann, Y.H. Chien, P.P. Jones, M.M. Davis, and H.M. McConnell. 1996. Early biochemical signals arise from low affinity TCR-ligand reactions at the cell–cell interface. *J. Exp. Med.* 184:777–782.

8. Valitutti, S., S. Muller, M. Cella, E. Padovan, and A. Lanzavecchia. 1995. Serial triggering of many T-cell receptors by a few peptide–MHC complexes. *Nature (Lond.).* 375:148–151.

9. Valitutti, S., and A. Lanzavecchia. 1997. Serial triggering of T-cell receptors: a basis for the sensitivity and specificity of T cell antigen recognition. *Immunol. Today.* In press.

10. Viola, A., and A. Lanzavecchia. 1996. T cell activation determined by T cell receptor number and tunable thresholds. *Science (Wash. DC).* 273:104.

11. Wülfing, C., J.D. Rabinowitz, C. Beeson, M.D. Sjaastad, H.M. McConnell, and M.M. Davis. 1997. Kinetics and extent of T cell activation as measured with the calcium signal. *J. Exp. Med.* In press.

12. Preckel, T., R. Grimm, S. Marin, and H.U. Weltzien. 1997. Altered hapten ligands antagonize trinitrophenyl-specific cytotoxic T cells and block internalization of hapten-specific receptors. *J. Exp. Med.* In press.

13. De Magistris, M.T., J. Alexander, M. Coggeshall, A. Altman, F.C. Gaeta, H.M. Grey, and A. Sette. 1992. Antigen analog–major histocompatibility complexes act as antagonists of the T cell receptor. *Cell.* 68:625–634.

14. Kersh, G.J., and P.M. Allen. 1996. Essential flexibility in the T-cell recognition of antigen. *Nature (Lond.).* 380:495–498.

15. Reis e Sousa, C., E.H. Levine, and R.N. Germain. 1996. Partial signaling by CD8$^+$ T cells in response to antagonist ligands. *J. Exp. Med.* 184:149–157.

16. Lyons, D.S., S.A. Lieberman, J. Hampl, J.J. Boniface, Y. Chien, L. J. Berg, and M.M. Davis. 1996. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. *Immunity.* 5:53–61.

17. Cai, Z., H. Kishimoto, A. Brunmark, M.R. Jackson, P.A. Peterson, and J. Sprent. 1997. Requirements for peptide-induced T cell receptor downregulation on naive CD8$^+$ T cells. *J. Exp. Med.* 185:641–651.

18. Schwartz, R.H. 1990. A cell culture model for T lymphocyte clonal anergy. *Science (Wash. DC).* 248:1349–1356.

19. Valitutti, S., S. Muller, M. Salio, and A. Lanzavecchia. 1997. Degradation of TCR/CD3-z complexes following antigenic stimulation. *J. Exp. Med.* In press.

20. Valitutti, S., S. Muller, M. Dessing, and A. Lanzavecchia. 1996. Signal extinction and T cell repolarization in T helper cell–antigen-presenting cell conjugates. *Eur. J. Immunol.* 26:2012–2016.