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Commentary

T cell receptor antagonism *in vivo*, at last

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The T cell receptor (TCR) for antigen recognizes a combination of a peptide antigen bound to a major histocompatibility complex molecule. Providing that the antigen is presented on a suitable cell, this interaction leads to stimulation of the T cell, inducing a series of biochemical events beginning with activation of tyrosine kinases and eventually leading to T cell proliferation and acquisition of effector functions, such as cytokine production (1). This pattern suggests a model in which the TCR acts as a simple trigger for T cell stimulation and that TCR occupancy equals T cell activation. Over the last few years however, it has become apparent that this cozy picture is incorrect and that, rather than an on/off switch for T cell responses, the TCR acts as a rheostat (or dimmer switch) and that different TCR ligands can lead to intermediate stages of T cell activation (for recent reviews, see refs. 2 and 3). Thus TCR ligands have been described, which induce only a subset of the typical T cell responses (e.g., cytokine release without proliferation) or inhibit T cell responses altogether. Borrowing from pharmacology, immunologists termed these suboptimal ligands TCR partial agonists or TCR antagonists, and, since most were generated by using variants of the antigenic peptide, these ligands also have been termed altered peptide ligands. Until recently, studies of these ligands was confined to *in vitro* assays, with teasing glimpses of possible *in vivo* roles (see below). In this issue, Basu *et al.* (4) describe TCR antagonism *in vivo*, demonstrating for the first time that such ligands expressed as self peptide/major histocompatibility complex ligands can and do impact the primary T cell response occurring in lymphoid tissue.

Their approach is based on tracking the responses of a small number of antigen-specific T cells bearing a defined TCR, a technique which has facilitated intricate analysis of T cell responses *in vivo* (5). T cells were introduced either into a normal mouse or into one in which all antigen-presenting cells express a potent, specific TCR antagonist (6). When activated after immunization with specific antigen, the T cells in the normal animal proliferate extensively in the appropriate lymph node. In the presence of the TCR antagonist, however, the proliferation was markedly impaired. Importantly, exposure to the antagonist ligand did not appear to adversely influence survival or migration of the T cells before stimulation. Previous work (6) indicated that this inhibition truly reflects TCR antagonism rather than, for example, some form of T cell anergy, another state that can be induced by certain suboptimal TCR ligands (2, 3).

Basu *et al.* (4) employed a model system in which they used transgenesis to express a synthetic TCR antagonist as a "self peptide" *in vivo*. However, there have been several reports of TCR antagonists occurring in nature—specifically generated by pathogens during the course of an infection. Early reports defined antigenic variants generated during infection with HIV (7, 8) or Hepatitis B virus (9), which behaved as TCR antagonists for the patients' own T cells. Similarly, recent reports describe potent, naturally occurring TCR antagonists in patients with malaria infections (10). These data have been taken to suggest that production of such variants may assist pathogen escape from immune recognition by inhibiting and/or deflecting the T cell response (8, 10).

A less sinister role for suboptimal TCR ligands has been proposed based on the capacity of both TCR antagonists and partial agonists to induce development of CD8+ "killer" T cells in the thymus (11–14). These data suggest that immature T cells, poised to differentiate, can respond to suboptimal TCR ligands that mature T cells ignore, a property directly demonstrated in recent experiments (15). Moreover, taken together with the current work by Basu *et al.* (4) that suboptimal TCR ligands can indeed inhibit primary T cell responses *in vivo*, these findings imply that at least some ligands which induce T cell development may be capable of regulating the mature T cell response –an interesting solution for an immune system obsessed with checks and balances. The subtlety of this model is that although TCR antagonists can block T cell responses, this typically requires considerable antagonist excess—indeed Basu *et al.* (4) mention that even in the presence of the endogenous TCR antagonist, a response to antigen can be achieved if sufficient antigen is administered (a feature which pharmacologists would recognize as surmountibility of antagonism).

On the other hand, it is not clear what role (if any) altered peptide ligands play in CD4 "helper" T cell development—recall that all examples of a positive role for altered peptide ligands in thymic development has been observed for CD8 T cells. Indeed, Allen and coworkers (6) previous work using their current system indicates that *in vivo* TCR antagonist expression has a mild effect on specific CD4 T cell development. Moreover, some reports indicate that suboptimal ligands may inhibit CD4 T cell development (16) or direct T cell development away from the CD4 and into the CD8 lineage (17, 18). This result is exciting because it may point to a fundamental difference in the requirements for development of CD4+ versus CD8+ T cells.

Whatever their physiological relevance, it is clear that suboptimal TCR ligands can profoundly influence T cell activation. How do they achieve this? The mechanism by which variant TCR ligands block or divert T cell responses is still unclear. One key feature, however, appears to be the capacity to induce weak and/or transient activation of the standard tyrosine phosphorylation cascade promulgated through the TCR: Specifically, there is evidence for partial tyrosine phosphorylation of the TCR-associated ζ-chain, a key target in the first stages of TCR-induced signal transduction (19–21). Other features of altered peptide ligands are less consistent between different systems. For example, in several well described cases, (including the system used in the current work—G. Kersh and P. Allen, unpublished data) the TCR affinity for suboptimal ligands has been found to be lower than that measured for the "full" agonist ligand. These differences in affinity can range from as little as 3-fold (22) to as much as 50-fold (23). Other groups contend that there is a poor correlation between TCR affinity and bio-activity (24). Some of these differences may

Abbreviation: TCR, T cell receptor.

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reflect another layer of complexity because most affinity measurements do not include the contribution of other T cell surface molecules, in particular the coreceptors (CD4 and CD8), which in some cases are capable of altering the TCR off-rate (25).

How might these features block or alter T cell responses? Allen and coworkers (26) have again supplied a tempting answer: in an recent report, they defined the step-wise phosphorylation of the six tyrosine residues on each TCR-ζ chain induced by TCR engagement (26). Because it will take time for phosphorylation of all of these sites, premature release of ligand by the TCR (e.g., by a fast TCR off-rate or inadequate coreceptor involvement) would leave the TCR-ζ chain phosphorylation in limbo—neither activated or unactivated. This event could simply incapacitate that individual TCR, preventing further participation in signaling. Alternatively, such partial activation could lead to a dominant negative or "spoiling" signal from the TCR, which would dampen activation of other TCRs in the same cell (2, 3). There is evidence for and against this latter hypothesis (B. Evavold, personal communication; M. Daniels and S.C.J., manuscript in preparation)—which answer holds true may depend on the precise agonist/antagonist properties of the ligand (see below).

Indeed, one advantage of the system described by Basu *et al.* (4) is that it can be used to analyze primary *in vivo* responses of other CD4 or CD8 T cells to suboptimal ligands. This may help clear up a persistent problem in comparing data on this topic from different labs: The criteria for defining the properties of a TCR ligand. A TCR antagonist may be defined by its capacity to block killing by a cytotoxic T cell clone in one laboratory or by inhibiting proliferation of naïve CD4 T cells in another. While both ligands may indeed operate by the same mechanism, it is equally plausible that they achieve these effects by different means, leading to their inappropriate grouping as "antagonist". Indeed, the system used by Basu *et al.* (4) is of this sort. Although these ligands fail to induce typical CD4 T cell responses (such as proliferation and cytokine production), they do allow these same T cells to kill tumor cells bearing the same variant peptide (27). To those of us who typically rely on cytotoxicity as a readout of TCR agonist interactions, these ligands would have a different classification. Thus, some of the inconsistencies between systems alluded to earlier may be based in subtle differences between the agonist/antagonist properties or the ligands tested, or the type of assays used to study T cell responses *in vitro*. On the other hand, the activation of naive T cells after antigenic challenge, albeit by using TCR transgenic cells, has the appeal of studying T cells in their natural environment interacting with natural antigen-presenting cells: Perhaps here at last is a more physiologically pertinent assay to define properties of suboptimal ligands on mature T cells.

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