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Strength of TCR–Peptide/MHC Interactions and In Vivo T Cell Responses

Emily Corse,*1 Rachel A. Gottschalk,*,†1 and James P. Allison*‡

The TCR can detect subtle differences in the strength of interaction with peptide/MHC ligand and transmit this information to influence downstream events in T cell responses. Manipulation of the factor commonly referred to as TCR signal strength can be achieved by changing the amount or quality of peptide/MHC ligand. Recent work has enhanced our understanding of the many variables that contribute to the apparent cumulative strength of TCR stimulation during immunogenic and tolerogenic T cell responses. In this review, we consider data from in vitro studies in the context of in vivo immune responses and discuss in vivo consequences of manipulation of strength of TCR stimulation, including influences on T cell–APC interactions, the magnitude and quality of the T cell response, and the types of fate decisions made by peripheral T cells.

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Stimulation of T cells in the context of in vitro culture systems has been crucial for defining the biochemical nature of TCR–pMHC interactions that influence the outcome of T cell activation. To understand how these parameters affect physiological T cell function, it is necessary to focus on evaluation of the effects of changing TCR–pMHC interactions within the context of in vivo immune responses. This is because of the limitations of in vitro assays in the evaluation of spatial and temporal aspects of in vivo T cell responses and the potential implications of in vivo studies for the design of more effective T cell vaccines that result in sustained T cell activity against pathogens and tumors.

Historical perspectives of TCR ligand strength

The mitogenic effects of monoclonal anti-CD3 Ab upon T cells were known even prior to molecular characterization of the TCR itself; early studies with human T cells and OKT3 Ab (4) showed that addition of increasing amounts of anti-CD3 resulted in increasing amounts of DNA synthesis as measured by incorporation of [3H]thymidine (5). Subsequent experiments provided early insight into how different amounts of TCR engagement are interpreted by T cells via intracellular signaling pathways (6). In the more than 30 years since the first use of anti-CD3 Ab, a large body of experiments involving stimulation of cultured T cell lines with anti-CD3 has defined the key signaling pathways of T cell activation (7). Engagement of TCR with Ab is orders of magnitude stronger than cognate recognition of pMHC (1). Thus, for understanding physiological TCR stimulation in terms of the qualitative and quantitative nature of peptide ligands presented by MHC, the relevance of TCR engagement with Ab is limited. In addition, reports describing the effects of a comparison of TCR signal strength are found throughout the literature to refer to changes in a wide variety of activating T cell stimuli, including changes in amount and quality of pMHC ligand, mutations in downstream signaling molecules, and even activation status of the APC. For the sake of clarity, this review will focus mainly on the variation of amount or quality of pMHC ligand.

The cloning of TCR genes and creation of transgenic mice expressing TCRs of known specificity (8, 9) allowed for the

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Abbreviations used in this article: DC, dendritic cell; $k_{on}$, dissociation rate; $k_{off}$, association rate; pMHC, peptide/MHC; TFH, T follicular helper cell.

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study of TCR engagement by physiological Ags and paved the way for investigation of the biochemical requirements of T cell activation through experimental modification of both pMHC ligand and TCR variable regions. Experiments with T cells of known specificity showed that the TCR is sensitive to single amino acid changes in the sequence of cognate peptide (8). Development of tetrameric complexes of pMHC (10) allowed for detection of rare Ag-specific T cells within polyclonal populations and also provided a complementary method to soluble binding assays for estimation of biochemical parameters of off-rate and apparent affinity (11).

Many studies that have examined the effects of qualitative changes in pMHC ligand over the past three decades involve the cytochrome c peptide Ag system for CD4+ T cells and the OVA peptide Ag system for CD8+ T cells. Although this is likely due at least in part to the arduous nature of defining MHC and TCR binding parameters, this field of study would benefit from the examination of more varied TCRs to assess the general applicability of the concepts learned from the systems mentioned above. The continued development of ligand panels for increasing numbers of TCRs is likely to enhance our understanding of how these parameters function in initial T cell activation and influence the outcomes of in vivo T cell responses.

**TCR–pMHC binding parameters: Definitions and controversies**

The interaction of TCR with pMHC ligand is described by the following equation:

\[ TCR + pMHC \xrightleftharpoons[kt]{k_{on}} \frac{TCR}{pMHC}. \]

Alterations in peptide ligand that result in better or worse binding to MHC are effectively a change in concentration of pMHC ligand. This becomes especially significant in in vivo settings, where encounter with Ag is usually more rare. When comparing different peptide ligands, careful determination of MHC binding is obviously essential prior to making conclusions as to the effect of various ligands upon TCR–pMHC interactions.

The rate at which TCR and pMHC associate (\(k_{on}\)) and dissociate (\(k_{off}\)) is shown. The \(t_{1/2}\) of the TCR–pMHC interaction is related to the off-rate \(k_{off}\) by the equation \(t_{1/2} = \ln(2) / k_{off}\). Thus, shorter TCR–pMHC interactions have shorter \(t_{1/2}\) and faster off-rates relative to longer TCR–pMHC interactions. In equilibrium conditions, the affinity is calculated from \(k_{off} / k_{on}\) which can be derived from surface plasmon resonance measurements. The \(t_{1/2}\) of TCR–pMHC interactions can also be measured by dissociation of fluorescently labeled pMHC tetramers (11), and the off-rates calculated from tetramer dissociation have been shown to correlate well with those derived by surface plasmon resonance (12, 13).

When evaluating the effects of the above biochemical parameters upon T cell responses, it is important to consider the variability in methods used to measure the extent of T cell activation. Even commonly used assays, such as quantitation of calcium flux, proliferation, and cytokine production exhibited by T cells in response to stimulation, are often carried out differently depending on the investigator or laboratory conducting the study. The problems that such variability causes for the interpretation of data gathered from assays of human T cells in international and multicenter immune-monitoring projects has been discussed (14), and the same concept also applies to more basic studies. It is thus essential to consider the type and quality of T cell assay before making conclusions about the T cell responses measured. For the purposes of this review, to consider results from multiple studies, we define the potency of T cell activation as how well a particular TCR stimulus induces a T cell response within a given assay. Thus, a higher-potency pMHC ligand activates T cells better than a lower-potency peptide ligand at the same concentration, and we will use these terms when the precise biochemistry behind the pMHC ligand potency (i.e., affinity or off-rate) has not been determined. Finally, references to the strength of TCR–pMHC interaction or TCR stimulation assume that stronger interaction or stimulation has a longer \(t_{1/2}\) or is of higher affinity, although in some cases this must be inferred in the absence of a direct demonstration.

Early studies comparing pMHC ligands with different off-rates of binding to TCR showed that this parameter can greatly influence the potency of T cell activation (15) and led to the model of a kinetic basis for TCR ligand discrimination known as kinetic proofreading (16). pMHC ligands with slower off-rates were subsequently shown to induce more extensive phosphorylation of CD3ε subunits, providing an important mechanistic connection between extracellular and intracellular events (17). In a transfection system of normal TCRs and TCRs engineered to have higher affinity for pMHC ligand, affinity was shown to have a strong relationship with T cell activity (18), and both affinity and off-rate of TCR–pMHC interactions have been heavily studied as important correlates of the extent of T cell activation by pMHC (19). Discrimination between different pMHC ligands by the TCR is likely to involve complex regulation of the activity of phosphatases and other signaling molecules in response to subtleties in the kinetic and equilibrium parameters of TCR engagement (20).

Reports of a correlation between measurements of the off-rate (17, 21, 22) or the affinity (18, 23, 24) for various TCR/pMHC pairs with potency of T cell activation led to an apparent discrepancy as to whether off-rate or affinity is the more important factor contributing to robust T cell stimulation (19). Recent insight into this problem was provided by a study that categorized a large panel of pMHC ligands for a tumor-reactive CD8+ TCR according to the association rate (25). For pMHC with faster association rates with TCR (>10^5 M^-1 s^-1), affinity is the better predictor of T cell activation potency, and for slower association rates (10^3 M^-1 s^-1), the off-rate of interaction is the closer correlate. This concept applies to a variety of CD4+ and CD8+ TCR–pMHC interactions, including those in the seemingly disparate studies mentioned above (25). However, it stands to reason that the multitude of TCR–pMHC ligand pairs in nature are likely to interact with a wide variety of biochemistries, and therefore the outcome of TCR ligation may be influenced by different biochemical and thermodynamic parameters depending upon the particular TCR–pMHC interaction (12).

Thus, consideration of TCR–pMHC binding according to any single parameter such as off-rate or affinity is likely to be limited in applicability. In addition, physical constraints imposed by the juxtaposition of the T cell and APC membranes almost certainly influence TCR binding to pMHC (26–28).
Indeed, recent fluorescence resonance energy transfer measurements between TCR and pMHC molecules on cellular membranes indicate that off-rate is much faster and affinity is much higher than when measured in solution (29). Also, examination of TCR–pMHC binding parameters using mechanical assays showed a correlation between T cell activation potency and the kinetic parameters of membrane-bound TCR–pMHC interactions but not those derived from soluble binding assays (30).

Early T cell activation events are often delayed in response to weak pMHC ligands

A common theme emerges in studies that have examined early T cell activation events in response to pMHC ligands with shorter (or lower affinity) interactions with TCR, but which still induce T cell proliferation: the events are delayed but often occur with comparable efficiency if given enough time. Increase in the concentration of intracellular Ca\(^{2+}\) occurs with a delay after a shorter TCR–pMHC interaction (31) or a lower dose of pMHC ligand (32). A recent study of Ca\(^ {2+}\) dynamics in tumor-reactive T cells showed that a high-affinity pMHC ligand induced sustained Ca\(^ {2+}\) flux and emptying of endoplasmic reticulum Ca\(^ {2+}\) stores, but oscillating Ca\(^ {2+}\) flux and only partial endoplasmic reticulum emptying was observed with a lower-affinity ligand during the time frame examined (33). In addition, recruitment of CD3\(\xi\) to the T cell–APC synapse was delayed in response to a pMHC ligand with a shorter TCR interaction, but normal levels of accumulation (as seen with natural pMHC ligand) were achieved after twice as much time had elapsed (34). Interestingly, the immunological synapse may function in the amplification of signals originating from shorter and lower-affinity TCR–pMHC interactions (35, 36).

In naive T cells, accumulation of signaling intermediates in response to pMHC ligands appears to be slower and more sustained than that observed after stimulation with anti-CD3 Ab (32, 37, 38). Although this may not be surprising due to the much greater affinity of the Ab compared with the pMHC ligand, a longer period of signal accumulation after initial pMHC encounter may be important for integration of the complex inputs a T cell receives prior to commitment to cell division and cytokine production (39). The observation of delayed but significant levels of phosphorylated c-Jun in response to a weaker CD8 T cell ligand led to the proposal that the accumulation of some signaling intermediates could function to quantify cumulative TCR signal (32). This may be particularly relevant during in vivo immune responses, where signals are likely to be integrated from T–APC contacts varying in stability and number (see below). The idea that responses to weaker TCR stimulation can catch up if given enough time is consistent with our in vivo finding that maximal expression of ki67 Ag occurs with a substantial delay in response to lower-affinity pMHC ligand (40).

Ag density sets thresholds for effector responses in vivo

In vivo T cell responses are triggered by TCR recognition of pMHC ligand presented by a dendritic cell (DC) or other APC. Naive T cells continuously scan the DC network for encounter of cognate Ag, upon which signaling can occur in the context of a variety of transient and/or stable interactions (41, 42). The nature of these T cell–DC interactions, as visualized by live imaging within lymphoid tissues, largely defines the outcome of the immune response, with prolonged stable interactions being required for effective proliferation and expression of effector molecules (42).

Comparison of interactions between naive CD8 T cells and DCs pulsed ex vivo with different amounts of peptide showed that more T cells clustered around DCs loaded with more peptide (43). In agreement with this finding, there is an inverse relationship between the time it takes naive CD8 T cells to form stable interactions and the concentration of antigenic peptide (44). Together these data suggest that higher pMHC ligand densities increase the chance that cognate T cells will be stopped by a given DC. Only concentrations of pMHC that induced stable interactions resulted in proliferation, and a 2-fold change in pMHC density caused the majority of responding cells to switch from nondividing to dividing (44). A similar sharp transition to proliferation has also been reported for in vivo pMHC dose responses of CD4 T cells (45). Thus, pMHC ligand density has an important influence on the duration and quality of in vivo T–DC interactions and therefore also in determining the threshold for in vivo T cell proliferation.

Interestingly, T cells form stable interactions more quickly with DCs expressing low amounts of peptide when high Ag density DCs are also present, showing that T cells integrate signals from multiple T–APC interactions (44). This highlights the complexity of factors likely to determine the level of

FIGURE 1. Variables influencing cumulative strength of TCR stimulation in vivo. When considering the quantity and quality of TCR–pMHC interactions in vivo, there are several levels of complexity that contribute to the strength of TCR stimulation: biochemical parameters of TCR–pMHC interactions, such as affinity and \(t_{1/2}\), density of pMHC ligand, and temporal components, including duration of T cell–APC interactions and Ag persistence.
cumulative TCR stimulation in vivo (Fig. 1). Integration of TCR stimulation from multiple pMHC encounters [whether from multiple T–APC contacts or from serial triggering within one contact (46)] could be important not only for achieving certain thresholds of stimulation during initial priming, but also throughout the expansion phase. Studies in which Ag was removed during in vivo T cell responses showed that prolonged Ag persistence is required for continued CD4+ T cell proliferation and effector differentiation (45) and that duration of Ag exposure influences the magnitude of initial expansion of CD8+ T cells, but has less impact on their effector function (47).

**Apparent TCR–pMHC affinity and T cell repertoire**

In addition to setting activation thresholds for individual T cell clones, pMHC ligand shapes the in vivo T cell response by influencing the TCR repertoire. By analyzing tetramer binding, it is possible to estimate the average affinity of TCR–pMHC interactions of responder T cells (referred to in this review as apparent affinity), although some measurements of tetramer intensity are influenced by factors other than affinity (48, 49). Studies examining TCR repertoire in primary and secondary immune responses observed greater intensity of tetramer staining during the secondary response, consistent with an increase in average TCR affinity of participating T cells (11, 50). During the progression of a diabetogenic T cell response, a selective advantage was observed for T cells expressing TCRs with a higher apparent affinity for pMHC ligands (51). Consistent with this, analysis of a pre- and post-immune TCR repertoire in mice expressing a fixed TCRβ-chain showed loss of clones that weakly bound tetramer (52). In a similar study, immunization with a peptide ligand that induced less expansion compared with the natural ligand (while binding comparably to MHC) resulted in greater pMHC binding intensity of the responding T cell population (53). An inverse relationship has also been described between peptide dose and apparent receptor affinity of responding T cells present several months after the primary response (54). Higher levels of tetramer staining after immunization with lower doses of Ag could indicate that limiting amounts of Ag set a threshold that favors preferential selection of cells with a high affinity for pMHC. Also, lower apparent TCR affinity observed in response to higher pMHC potency could be caused by inhibition or deletion of highest-affinity responder T cells, which could be important in the prevention of pathogenic immune responses (51). Such results have also been reported upon immunization with peptide exhibiting higher binding affinity for MHC, thus resulting in an increased density of pMHC complexes (55). In comparison with the natural ligand, immunization with this altered peptide resulted in a homogeneous population of responder T cells with a lower affinity for pMHC, due to elimination of T cells bearing high-affinity receptors by apoptosis. Collectively, these studies suggest that pMHC density and potency may set thresholds at either end of the TCR affinity spectrum, which results in skewing of the repertoire to yield an optimal TCR affinity of responding T cells for their cognate Ag.

Infection with either *Listeria monocytogenes* or lymphocytic choriomeningitis virus expressing the same antigenic epitope results in a differential Ag sensitivity of responding T cells, as measured by IFN-γ production (56). Although there are many variables that influence responsiveness of T cells to Ag (57), the data highlight the potential relevance of changes in apparent TCR affinity of a polyclonal T cell repertoire during infection. Thus, the clonal diversity and apparent TCR affinity of a polyclonal T cell repertoire could be influenced by a combination of loss of clones failing to achieve stimulation above a threshold necessary for activation, overrepresentation of higher-affinity clones having a proliferative advantage during the expansion phase, and deletion or inhibition of the highest-affinity clones due to overstimulation.

**TCR–pMHC interaction parameters and in vivo T cell immunity**

T cells present in polyclonal repertoires exhibit diverse TCR–pMHC binding parameters for a given pMHC ligand. The selective expansion of T cells bearing a particular TCR of unknown affinity during an immune response highlights the importance of TCR–pMHC interactions in repertoire selection, but does not allow for a more precise understanding of how particular determinants of these interactions influence the magnitude and quality of T cell activation in vivo. It is necessary to study responses of a monoclonal population of T cells to pMHC ligands varying in potency to assess the influence of this variable on multiple stages of the immune response.

The influence of pMHC ligand potency upon in vitro T cell proliferation, cytokine production, and cytotoxic activity is documented (15, 58). The relationship between TCR–pMHC interaction kinetics and intracellular signaling to cytotoxic granule release was recently clarified in a study that observed efficient centrosome polarization but a decrease in granule recruitment in response to a shorter TCR–pMHC interaction (59). Lower-affinity pMHC ligand also correlates with less cytotoxic granule polarization in tumor-reactive T cells (33). In vivo, studies varying the amount of Ag showed a correlation between pMHC density and CD8+ cytotoxic function (44, 60). More Ag also correlated with a larger memory population (61, 62), though the highest levels of Ag had a detrimental effect on the number of IFN-γ-producing memory cells (61). Similar to greater Ag persistence, increased amount of initial Ag appears to enhance the magnitude of response rather than changing the pattern of effector or memory differentiation (63). However, a TCRβ mutant deficient in protein kinase C-θ localization and NF-κB signaling generated normal CD8+ effector responses but impaired memory differentiation (64), showing that differential signaling through the TCR is likely to contribute to effector and memory formation.

A recent study of in vivo progression of a monoclonal CD8+ T cell response to *Listeria* demonstrated that relatively low-affinity pMHC ligands could induce expansion and generation of functional cytotoxic and memory T cells (65). Across the panel of six pMHC ligands examined, pMHC affinity influenced both the amplitude and kinetics of the response, such that lower-affinity TCR–pMHC interactions resulted in an earlier peak in accumulation of Ag-specific T cells, as well as their earlier exit from lymphoid organs. Our study of a CD4+ T cell response to peptide immunization showed similar results in that the lowest-affinity pMHC ligand induced proliferation, cytokine production, and memory formation, though to a smaller degree than higher-affinity peptides (66).
Despite predictions from in vitro T cell proliferation assays, we observed attenuated CD4⁺ T cell responses in vivo to a pMHC ligand with a slow off-rate. Immunization with this pMHC ligand, which interacts longer with TCR than the corresponding natural ligand, resulted in decreased proliferation, cytokine production, and memory formation, and this correlated with decreased levels of intracellular signaling intermediates (66). These data suggest the existence of an upper limit to increased responsiveness to long-lived (or high-affinity) TCR–pMHC interactions in vivo, which could have implications for vaccine design involving high-affinity pMHC ligands. Vaccination with pMHC ligands of intermediate affinity for a CD8⁺ tumor-reactive TCR elicited the best tumor-free survival, which correlated with in vivo responses in a related polyclonal T cell repertoire (23). Thus, in vivo pMHC ligand potency is not necessarily equivalent to that measured in vitro, and it is important to evaluate of the in vivo efficacy of potential vaccine ligands.

Strength of TCR stimulation and peripheral CD4⁺ T cell fate

Naive CD4⁺ T cells are capable of differentiation into several lineages upon TCR engagement (67). In vitro evidence indicates that pMHC ligands with relatively fast off-rates from TCR induce IL-4 production, which suggests that weaker TCR signals favor Th2 differentiation (68), although consideration of dose and timing highlights complexity in this process (69). IL-4 production can also be achieved with low doses of natural pMHC ligand (70); Erk activity is thought to inhibit IL-4 synthesis by T cells stimulated with high doses of peptide (71, 72). In vivo, it is likely that an LPS-induced inflammatory environment overrides the ability of low-affinity pMHC ligands to induce Th2 responses (66). However, in less polarizing conditions, the relevance of low-affinity TCR engagement to in vivo Th2 responses is suggested by a recent study that found increased IL-4 production by a polyclonal T cell population after removal of clones that react with high affinity to pMHC ligand (73). Low amounts of Ag could also be favorable for Th2 skewing in vivo; it is interesting to speculate that differential MHC expression could affect T cell polarization by various types of APC.

Within a polyclonal repertoire, it is possible that the biochemical properties of individual TCRs may direct T cells toward particular differentiation pathways. In TCRβ transgenic mice expressing endogenous ligand, weakly tetramer-positive T cells that escaped negative selection were more prone to IL-4 production upon ex vivo stimulation (73). In the case of T follicular helper cells (Tfh), monoclonal T cells with higher affinity for Ag differentiate into Tfh more efficiently, and in polyclonal responses, Tfh have stronger tetramer binding than non-Tfh CD4⁺ T cells (53).

In the absence of inflammatory stimuli, low doses of pMHC ligand favor conversion of naive CD4 T cells into Foxp3⁺ regulatory T cells (74, 75). In short-term in vitro assays, pMHC ligands of varying potency result in Foxp3 induction if the dose is altered to compensate for potency, such that sufficiently weak TCR stimulation is achieved (40, 76). However, when we compared pMHC ligands of varying affinity in vivo, we found that only relatively high-affinity TCR–pMHC interactions facilitated induction of persistent Foxp3⁺ T cells, and increasing ligand density could not compensate for low-affinity pMHC ligands (40). Thus, in vivo data suggest that not all TCR–pMHC interactions may be conducive for differentiation toward every potential Th fate.

pMHC ligand potency, density, and peripheral tolerance

The potency of pMHC ligand can dictate the stability of T–DC interactions and thus the degree of proliferation (77). However, in this setting of tolerance, pMHC ligands of varying potencies were all able to induce T cell anergy, even in the absence of detectable stable contacts in the case of the lowest-potency ligands. The anergy induced by the lowest-potency ligand was coincident with upregulation of CD69, downregulation of CD62L, and retention in the lymph node. This retention likely increased the chances of continued Ag encounter, which may be important for signal integration during transient T–DC contacts. Anergy driven by high- but not low-potency TCR stimulation was calcineurin dependent, suggesting that T cell interpretation of low-affinity pMHC ligands that fail to induce stable interactions may occur through a distinct biochemical mechanism.

In a model of tolerance to self-Ag, T cell expansion was proportional to Ag density, but all levels of Ag resulted in a state of tolerance (78). In this setting, higher Ag density resulted in a greater impairment of proliferation, cytokine production, and TCR signaling. These results are consistent with a study that varied Ag persistence, which indicated that prolonged TCR stimulation can become inhibitory as early as 10 d after initial Ag exposure (79). In the presence of inflammatory stimuli, induction of tolerance may be more dependent on Ag density, as low doses of Ag were required for induction of anergy in peripheral memory CD4 T cells (80).

TCR ligand density and affinity can also influence the route by which tolerance is achieved. We found that low doses of a higher-affinity pMHC ligand resulted in induction of a persistent population of Foxp3⁺ regulatory T cells, whereas high doses of a lower-affinity pMHC ligand resulted in deletion of all responding cells (even though both conditions induced comparable initial T cell proliferation) (40). These data demonstrate that T cells can distinguish between weak TCR stimulation achieved by reduced quantity versus quality of ligand. Collectively, the abovementioned studies suggest that a variety of TCR–pMHC interactions can result in tolerance, but that pMHC potency, density, and persistence may determine whether responding T cells express Foxp3, are deleted, or are rendered anergic.

Conclusions

Many variables influence how T cells perceive the strength of TCR stimulation during in vivo immune responses. Besides TCR–pMHC binding parameters, rare encounters with Ag-bearing APC make stability of pMHC complexes a critical factor, and prolonged T–APC interactions and Ag persistence within lymphoid organs add temporal components that may be less apparent in in vitro systems. Thus, it is important to consider the influence of TCR signal strength on various aspects of the in vivo immune response. Although in vitro data would suggest that stronger TCR–pMHC interactions always lead to more efficient T cell responses, in vivo there are likely to be additional constraints on both high and low levels of T cell stimulation. It is reasonable to speculate that attenuation of strong T cell responses in vivo may serve to pro-
tect the animal from detrimental levels of inflammation. Thus, there may be an optimal intermediate TCR affinity in polyclonal populations of T cells responding to a particular Ag. Given an appropriate time lag, many low-potency pMHC ligands can elicit comparable levels of T cell activation as their higher potency counterparts. Slow and sustained accumulation of signaling intermediates appears to be a feature of pMHC ligation of TCR (versus anti-CD3) and stimulation of naive T cells (as opposed to Ag-experienced or memory T cells). It is interesting to consider these observations in terms of the prolonged contact times observed by two-photon microscopy during in vivo T–DC interactions. This time period may be important for integration of the multiple inputs T cells receive prior to division and differentiation. During transient interactions, resulting from either low-density or low-potency pMHC ligand, integration of signals may take during the course of multiple encounters with APCs.

Perhaps the biggest challenge in understanding the in vivo consequences of differences in the strength of stimulation through the TCR is to extrapolate findings based on the study of monoclonal T cell populations to diverse polyclonal populations in which the fate of cells bearing particular TCRs are difficult to track. Considering the vast quantity of TCR specificities, one can imagine a wide variety of outcomes within the same immune response. In the case of differentiation, variability in TCR–pMHC interactions according to specificity or even slight differences in the number of available pMHC complexes could determine which clones form memory or differentiate into specialized subsets. The independent variables contributing to cumulative TCR stimulation, such as the biochemistry of TCR–pMHC interactions or the stability of pMHC, are not necessarily interchangeable, and understanding the distinct influences they may have is important to further the understanding of in vivo T cell responses.

Disclosures

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