Kinetic discrimination in T-cell activation

(T-cell receptor/antigen/major histocompatibility complex/reaction mechanism/antagonism)

JOSHUA D. RABINOWITZ*, CRAIG BEESON*, DANIEL S. LYONS†, MARK M. DAVIS†, AND HARDEN M. MCCONNELL*‡

*Department of Chemistry and †Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305

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ABSTRACT We propose a quantitative model for T-cell activation in which the rate of dissociation of ligand from T-cell receptors determines the agonist and antagonist properties of the ligand. The ligands are molecular complexes between antigenic peptides and proteins of the major histocompatibility complex on the surfaces of antigen-presenting cells. Binding of ligand to receptor triggers a series of biochemical reactions in the T cell. If the ligand dissociates after these reactions are complete, the T cell receives a positive activation signal. However, dissociation of ligand after completion of the first reaction but prior to generation of the final products results in partial T-cell activation, which acts to suppress a positive response. Such a negative signal is brought about by T-cell ligands containing the variants of antigenic peptides referred to as T-cell receptor antagonists. Results of recent experiments with altered peptide ligands compare favorably with T-cell responses predicted by this model.

In this paper we propose a model for both positive and negative triggering of T lymphocytes with special reference to the responsiveness of T-helper cells to T-cell receptor ligands on the surfaces of antigen-presenting cells. These ligands are molecular complexes of class II major histocompatibility complex (MHC) proteins and antigenic peptides. The background of this model is drawn from a number of sources.

(*i*) Cell–cell recognition often involves weak but specific forces (1, 2). Measurements of the binding of isolated T-cell receptors and their ligands show that this binding is weak (3–5), with rapid dissociation of the ligand from T-cell receptor ($t_{1/2}$ between 2 and 30 s at 25°C) (6, 7).

(*ii*) It is known that relatively few (50–200) ligands are sufficient to activate T cells (8–10). Lanzavecchia and coworkers (11) have demonstrated that a small number of ligands are sufficient to down-regulate a large number of T-cell receptors. These results suggest that each ligand interacts sequentially with a large number of T-cell receptors, a process referred to as serial engagement.

(*iii*) It has been discovered that when certain antigenic peptides are mutated, the mutated peptide may act as an antagonist, inhibiting T-cell responses to the antigenic peptide (ref. 12; reviewed in ref. 13). This inhibition is T-cell receptor specific and cannot be explained by competition for peptide binding to the MHC protein.

(*iv*) Activation of T cells by agonist and antagonist ligands results in different intracellular biochemical events. Agonist activation is associated with complete T-cell receptor phosphorylation and activation of the tyrosine kinase ZAP-70, whereas antagonist activation is associated with partial T-cell receptor phosphorylation and failure to activate this kinase (14, 15). In our discussion, the term T-cell receptor refers to the receptor molecule itself (an $\alpha\beta$ heterodimer), as well as the

associated proteins (the $\zeta\zeta$ homodimer, CD3 protein, and related kinases).

(*v*) Kinetic proofreading is a model in which the specificity of formation of a biochemical product is enhanced when the product is formed via a number of chemical steps, each of which tests the fidelity of a reaction intermediate (16). Mc-Keithan (17) has proposed that because of kinetic proofreading, T-cell activation may depend strongly on the rate of dissociation of ligand from T-cell receptors. In this paper we expand on this idea by proposing that incomplete receptor activation is inhibitory and that antagonist ligands cause incomplete receptor activation.

Kinetic Discrimination Model

Consider the simplest possible kinetic model. A schematic depicting the essential components of this model is shown in Fig. 1. Assume a number of ligands on the surface of the antigen-presenting cell that is small (e.g., 100) compared to the number of T-cell receptors on the surface of a T cell (1–5 × 10^4). In a steady-state condition, ligand L binds to and dissociates from the T-cell receptor (R),

$$L + R \underset{k_d}{\overset{k_{on}[R]}{\rightleftharpoons}} LR.$$
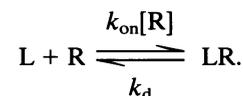
On forming the complex, the first intracellular biochemical event takes place rapidly to produce a covalent modification denoted LRP_1,

$$LR \overset{k_1}{\longrightarrow} LRP_1.$$
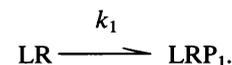
At this point, one of two events is possible. The receptor may be modified further, producing a second product (Fig. 1*A*),

$$LRP_1 \overset{k_2}{\longrightarrow} LRP_2,$$
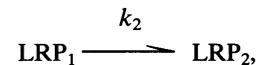
or the ligand may dissociate (Fig. 1*B*),

$$LRP_1 \overset{k_d}{\longrightarrow} RP_1 + L.$$
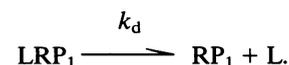
In this simple model, we postulate that the modified receptor RP_1 leads to a negative (incomplete) signal, whereas the modified receptor RP_2 leads to a positive (complete) signal. The overall scheme is thus,

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$$L + R \underset{k_d}{\overset{k_{on}[R]}{\rightleftharpoons}} LR \xrightarrow{k_1} LRP_1 \xrightarrow{k_2} LRP_2.$$

$$LRP_1 \downarrow k_d \qquad LRP_2 \downarrow k_d$$

$$RP_1 \qquad\qquad RP_2$$
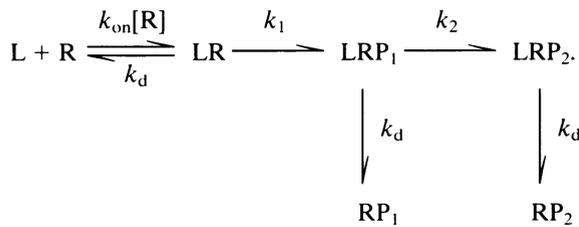
Scheme I

In Scheme I, it is assumed for simplicity that the rate of dissociation of ligand from the T-cell receptor (k_d) is independent of receptor activation state. Measurements of the dissociation of ligands from isolated T-cell receptors suggest that the rate constant k_d is between 0.02 s^{-1} and 0.05 s^{-1} for agonist ligands at $25°C$ (6, 7). While this rate constant will be increased at the physiological temperature (37°C), this may be offset by the effects of coreceptor molecules.

Also, from kinetic Scheme I, it is clear that under steady-state conditions the relative proportions of RP_1 and RP_2 are simply determined by the relative values of k_d and k_2. This steady-state approximation assumes serial engagement of T-cell receptors (11). The potential for serial engagement to enhance the statistical accuracy of the cellular activation signal is discussed in the *Appendix*.

One potential modification to the pathway proposed here (Scheme I) is that after dissociation of ligand from the T-cell receptor, another ligand may bind to the same receptor, enhancing the generation of RP_2. While the vast excess of receptor relative to ligand makes this unlikely at low ligand concentrations, at higher concentrations rebinding may be significant.

Given the large number of phosphorylation sites on the T-cell receptor complex, it is likely that receptor activation involves not one intermediate as depicted here (Scheme I) but

a large number of intermediates. Multiple intermediates could enhance the fidelity of kinetic discrimination by allowing for a greater number of editing steps (17). We model the effects of differing dissociation rates on the relationship between positive and negative signals in Fig. 2. An important feature of our model is that any ligand sends both positive and negative signals. Note that while additional proofreading steps somewhat reduce the overlap between positive and negative signals, they also reduce the signal amplitude.

Experimental Relevance

One of the attractive features of this model is the expected sensitivity of a T cell's response to changes in peptide structure. It is known that even the most conserved single amino acid replacements can convert an agonist ligand into an antagonist ligand (12). Recent measurements have shown that single amino acid changes in an antigenic peptide can affect the rate of dissociation of ligand from isolated T-cell receptors (ref. 7; D.S.L., S. A. Lieberman, J. Hampl, J. J. Boniface, Y. Chien, L. J. Berg, and M.M.D., unpublished results). These measurements have revealed that stronger agonists dissociate less rapidly than weaker agonists (7) and that antagonist peptides have even faster dissociation rates (D.S.L. *et al.*, unpublished results).

If a general property of antagonist ligands is that they dissociate more rapidly from the T-cell receptors, then they will serially engage a greater number of T-cell receptors (Fig. 2) (18). This effect may account for the ability of some antagonist ligands to block T-cell activation even when agonist is in excess (19, 20). We were interested in examining if the relationship between positive and negative signals and ligand dissociation rate shown in Fig. 2 could explain literature data on the physiologic effect of antagonist peptides. To investigate this issue, we related physiological T-cell activation to a simple

FIG. 1.   Schematic depiction of an interaction between a T-cell receptor and its ligand, a molecular complex of antigenic peptide and MHC protein on the surface of an antigen-presenting cell. Binding of a ligand to the T-cell receptor may result in an intracellular biochemical reaction resulting in a covalent modification of the T-cell receptor, producing the intermediate LRP_1. (*A*) Reaction of LRP_1 to produce LRP_2 causes positive T-cell activation. (*B*) Dissociation of the ligand from the receptor prior to production of LRP_2 results in partial T-cell activation, which acts to suppress a positive response.

FIG. 2.   Dependence of generation of positive and negative signals on T-cell receptor–ligand dissociation rate. Signals generated based on reaction Scheme I are shown as solid lines. The effect of doubling the number of editing steps is shown as dashed lines. Increasing average lifetime of ligand–receptor complex ($1/k_d$, x axis) is related to increasing affinity of T-cell receptor–ligand interaction. Positive and negative signals are related to three factors: the number of binding events per ligand per time (B), the fraction of ligand-bound T-cell receptor that reacts to form LRP_1 (f), and the fraction of LRP_1 that reacts to form LRP_2 (p) versus the fraction of LRP_1 that fails to react prior to ligand dissociation (q). The number of binding events is based on a steady-state approximation of the serial engagement model (11). Quantitatively $B = 1/(D + 1/k_d)$, where $D = 1/(k_{on} \cdot$T-cell receptor concentration). Based on reaction Scheme I, $f = k_1/(k_1 + k_d)$, $p = k_2/(k_2 + k_d)$, and $q = 1 - p = k_d/(k_2 + k_d)$. The fraction of binding events leading to LRP_2 is fp and the fraction of binding events leading to LRP_1 but not LRP_2 is $f \cdot q$. Plotted in solid lines are positive and negative signals per ligand per time ($P = B \cdot fp$ and $Q = B \cdot fq$, respectively). Dashed lines represent $B \cdot (fp)^2$ and $B \cdot (fq)^2$. Constants are $k_1 = 1$, $k_2 = 0.1$, and $D = 5$.

function of both the absolute number of positive signals and the ratio of positive and negative signals received by the T cell (Fig. 3 legend).

We tested the ability of this function to explain two important literature observations about T-cell receptor antagonism. The first observation was that increasing concentrations of agonist ligands can overcome antagonist inhibition (21). In Fig. 3*A* we model the effect of a fixed concentration of antagonist ligand on T-cell response to agonist ligand. The calculated antagonist inhibition curves shown in Fig. 3*A* have the appearance of classical antagonist inhibitions, but here antagonism results from incomplete receptor activation rather than direct competition for receptor binding.

The second observation was that some of the antagonists that are most effective at blocking T-cell activation at low concentrations are themselves agonists at higher concentrations (20, 21). The existence of such mixed agonist/antagonist
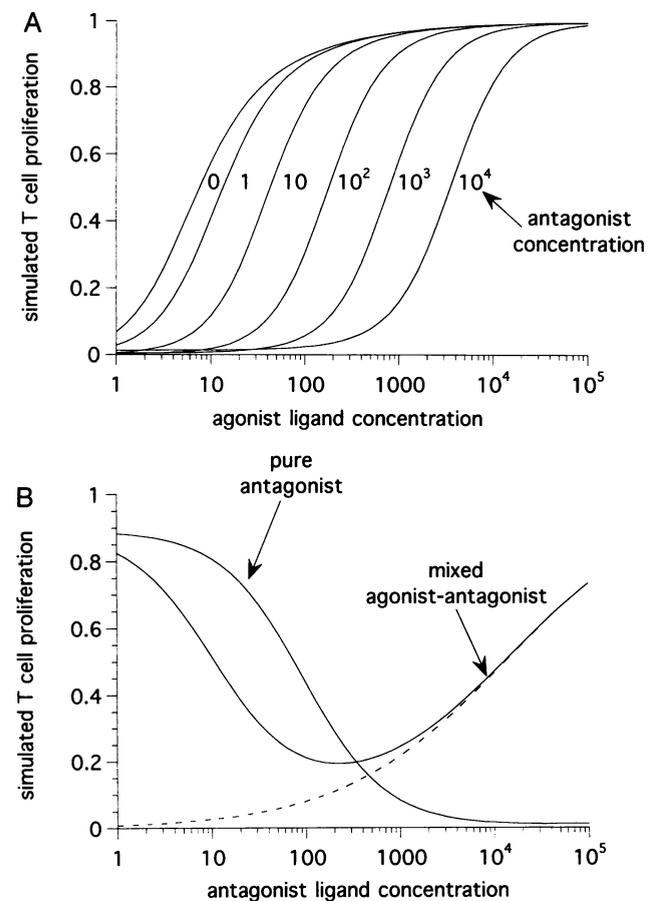
FIG. 3. Simulated T-cell proliferation in response to agonist and antagonist ligands. (*A*) Response to increasing concentrations of agonist ligand (k_d = 0.01) in the presence of fixed amounts of antagonist ligand (k_d = 10). Compare the predicted response depicted here with experimental results shown in figure 1 of ref. 21. (*B*) Antagonism of proliferation in response to a fixed concentration (100 units) of agonist ligand (k_d = 0.01) by increasing amounts of a mixed agonist-antagonist (k_d = 1) or pure antagonist (k_d = 20) ligand (solid lines). Predicted response to mixed agonist-antagonist ligand in the absence of agonist ligand is also shown (dashed line). Compare the predicted responses to figures 1 and 3 of ref. 20. Simulated T-cell proliferation curves were generated as follows: P_i and Q_i were computed as in Fig. 2 for given ligand *i* with dissociation rate k_{di}. The total number of positive (P_T) and negative (Q_T) signals received by the T cell is $P_T = \Sigma\ (C_i \cdot P_i)$ and $Q_T = \Sigma\ (C_i \cdot Q_i)$, where C_i is the concentration of ligand *i*. T-cell proliferation was modeled as $S = R \cdot (P_T)^\alpha$ where $R = P_T/(Q_T + \beta)$. Note that R depends primarily on the ratio of positive and negative signals. Graphed is a simple saturable function of S, $G = S/(1 + S)$. Constants are α = 0.5 and β = 0.01.

peptides follows logically form a model where every ligand sends some positive and some negative signals. As shown in Fig. 3*B*, the physiologic response to these peptides is accurately described by our simple function for predicting T-cell response (Fig. 3*B*). The shift from antagonist to agonist function with increasing concentration of a T-cell receptor ligand might also be related to rebinding of a ligand to a partially activated T-cell receptor.

Both the rate of dissociation of ligand from T-cell receptor, k_d, and the rate of intracellular biochemical reactions, k_2, may be affected by T-cell type (e.g., naive lymphocyte vs. differentiated T cell vs. hybridoma). In particular, k_d may be sensitive to changes in coreceptor and adhesion molecule expression. Recent results suggest that the coreceptor proteins CD4 and CD8 significantly affect the kinetics of T-cell receptor–ligand interaction, with CD8 expression resulting in a 10-fold reduction in ligand dissociation rate in one experiment (22). An example of the biological significance of ligand stabilization by coreceptor is an experiment in which expression of the extracellular domain of the CD4 protein in a CD4-negative hybridoma converted some antagonist T-cell receptor ligands into agonist ligands (23).

The rate at which intracellular biochemical reactions occur, k_2, will likely depend on the number and distribution of kinases intracellularly. Weiss and colleagues (24) have shown that the intracellular location of the tyrosine kinase ZAP-70 depends on T-cell type. In light of such data, it is not surprising that a given ligand may function as an agonist for one T cell but an antagonist for a different T cell with identical T-cell receptors (ref. 25; D.S.L. *et al.*, unpublished results). The ability of different levels of CD8 expression or monoclonal antibodies against CD8 to modulate the agonist and antagonist properties of some T-cell receptor ligands may involve changes in both k_d and k_2 (26).

Both positive and negative selection in the thymus are required for the development of a functional T-cell repertoire. Positive selection probably acts in part to ensure that T cells released into the periphery express a functional T-cell receptor. Negative selection is required to prevent the maturation of autoreactive T cells. The qualitatively different response of developing thymocytes to high- and low-affinity ligands (27) is most easily explained by a model where these ligands send qualitatively different signals. Kinetic discrimination is a simple mechanism to distinguish accurately T cells that are required to mount a functional immune response from those that might cause autoimmune disease.

Alternative Models

Many models for T-cell activation have been proposed, some of which attempt to account for the difference between agonist and antagonist ligands (as recently reviewed in refs. 13 and 28). The simplest model proposes that T-cell activation is governed by the affinity of T-cell receptor–ligand interaction, with antagonists competitively inhibiting the binding of agonist ligand to T-cell receptor. The small number of agonist ligands required for T-cell activation (8–10), the ability of some antagonist ligands to block activation even when agonist is in excess (19, 20), and the fast dissociation rate of antagonist ligands (D.S.L. *et al.*, unpublished results) argue against such a possibility.

Experiments using artificial ligands (such as monoclonal antibodies against the T-cell receptor) to activate T cells (29–31) have suggested two alternative mechanisms of T-cell activation. The first of these takes into account the importance of sufficient T-cell receptor oligimerization for T-cell responses (29, 30, 32). The importance of oligomerization suggests that antagonist ligands might block proper assembly of T-cell receptor dimers or oligimers (33). The ability of some antagonist ligands to block T-cell activation by superantigen

(34), or when agonist and antagonist ligands are presented on two separate antigen-presenting cells (19), would seem to rule out such a model.

The second type of model is based on results which suggest that only agonist ligands can induce the proper conformation of the T-cell receptor for full T-cell receptor activation (31). In such a model, antagonist ligands induce a distinct conformation of the receptor, which causes a negative signal (35). The observation that one T-cell line may generate an agonist response to a particular ligand, while another T-cell line expressing the identical T-cell receptor may generate an antagonist response to the same ligand, conflicts with this view (ref. 25; D.S.L. *et al.*, unpublished results).

Discussion

Chemically similar ligands can produce a large range of T-cell responses. Alterations as small as a single methyl group can change a peptide from one that causes T-cell stimulation into one that blocks it (12). Subtle variations in peptide structure can also produce ligands that induce long-lasting T-cell anergy (36) or that cause a shift from a Th1 to Th2 type cytokine profile (37, 38). These properties have resulted in great interest in altered peptides as potential pharmaceutical agents against autoimmune disease.

In this paper we propose a simple physical mechanism, kinetic discrimination, to account for the sensitivity of T-cell response to ligand structure. Kinetic discrimination postulates that ligands that bind to the T-cell receptor for an extended duration result in positive T-cell signaling, whereas ligands that bind only briefly result in negative T-cell signaling. In this model, as in kinetic proofreading, production of the final product is highly dependent on the dissociation rate of ligand from receptor. Kinetic discrimination differs from kinetic proofreading by relating biological response to a ratio of complete (positive) and incomplete (negative) signals. This allows kinetic discrimination to distinguish antagonist and agonist peptides in a single editing step (Figs. 2 and 3), in contrast to the large number of slow steps required to discriminate between ligands with similar dissociation rates in kinetic proofreading (17). Kinetic discrimination is also consistent with a large amount of experimental data on T-cell receptor antagonism. These data include direct measurements of the rate of dissociation of isolated agonist and antagonist ligands from the T-cell receptor in solution (D.S.L. *et al.*, unpublished results).

A critical question raised by this kinetic discrimination model is, how exactly could incomplete receptor activation suppress a positive response? One possibility is that antagonist receptor activation (production of RP_1) directly inhibits agonist receptor activation (LRP_2 generation). An example of a mechanism of this type would be binding of RP_1 to an enzyme required for generation of LRP_2. Data showing that some antagonist peptides can block calcium flux support a model where at least some antagonist peptides inhibit early T-cell activation (39).

Another possibility is that incomplete receptor activation results in production of some, but not all, required second messengers. For example, antagonist binding may generate second messenger A, whereas agonist binding generates second messengers A and B. Different T-cell responses (proliferation, cytokine production, and cytolysis) might differ in their sensitivity to the ratios of these second messengers. Data showing that some antagonists block interleukin 2 (IL-2) but not IL-3 production (40), whereas some agonists cause IL-4 but not IL-2 production (37), support a model of this type. It is likely that a full description of T-cell antagonism may incorporate elements of both of these mechanisms.

A satisfactory model of the intracellular events involved in positive and negative T-cell signaling will require significant progress in elucidating both the early second messenger pathways activated by antagonist ligands and the role of different second messengers in regulating gene transcription. The kinetic discrimination model we propose here may provide a framework to relate the kinetics of T-cell receptor–ligand interaction to these more complex events.

Appendix

For simplicity, the kinetic discrimination model presented in the text addresses only the average number of positive and negative signals per ligand per time (Fig. 2). However, this model can easily be generalized to describe the statistical probability of achieving a particular number of positive and negative signals in a given time. Assume that under steady-state conditions ligand L reacts to produce the complex LRP_1 N times. If the reaction pathway $LRP_1 \rightarrow LRP_2$ depends on a single molecular event, such as a phosphorylation step, the probability for this pathway is $p = k_2/(k_2 + k_d)$, and the probability of the dissociation pathway is $q = 1 - p = k_d/(k_2 + k_d)$. Out of the N times that the complex LRP_1 is formed, the probability $W(P)$ that LRP_2 will be formed P times is

$$W(P) = C(N,P)p^P q^{(N-P)}, \text{ where } C(N,P) = N!/(N - P)!P!$$

Note that this probability is sensitive to the relative values of p and q (k_2 and k_d) when N is large.

The physiologic response of the cell depends on the number of positive signals (P) versus negative signals (Q) received in a given time. The Gaussian probability distributions for positive versus negative signals vary as $\exp[(P - pN)^2/(2pqN)]$ and $\exp[(Q - qN)^2/(2pqN)]$, respectively. One sees that the positive and negative signal probability distributions are well separated as long as p and q (k_2 and k_d) are quite different, but that these probability distributions overlap strongly when p is similar to q. For a given p and q, the two populations become better discriminated as N increases, since the width of these distributions vary as $N^{1/2}$. Thus, serial triggering of a large number of T-cell receptors over an extended time period enhances the accuracy of discrimination between agonist and antagonist ligands. This is particularly important when the number of ligands on the antigen-presenting cell is small and when k_d is similar to k_2.

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