Calculations Show Substantial Serial Engagement of T Cell Receptors

Carla Wofsy,* Daniel Coombs,[†] and Byron Goldstein[‡]

*Department of Mathematics and Statistics, University of New Mexico, Albuquerque, New Mexico 87131; [†]Program in Applied Mathematics, University of Arizona, Tucson, Arizona 85721; and [‡]Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545 USA

ABSTRACT   The serial engagement model provides an attractive and plausible explanation for how a typical antigen presenting cell, exhibiting a low density of peptides recognized by a T cell, can initiate T cell responses. If a single peptide displayed by a major histocompatibility complex (MHC) can bind, sequentially, to different T cell receptors (TCR), then a few peptides can activate many receptors. To date, arguments supporting and questioning the prevalence of serial engagement have centered on the down-regulation of TCR after contact of T cells with antigen presenting cells. Recently, the existence of serial engagement has been challenged by the demonstration that engagement of TCR can down-regulate nonengaged bystander TCR. Here we show that for binding and dissociation rates that characterize interactions between T cell receptors and peptide-MHC, substantial serial engagement occurs. The result is independent of mechanisms and measurements of receptor down-regulation. The conclusion that single peptide-MHC engage many TCR, before diffusing out of the contact region between the antigen-presenting cell and the T cell, is based on a general first passage time calculation for a particle alternating between states in which different diffusion coefficients govern its transport.

INTRODUCTION

The activation of a T cell begins with the formation of an "immunological synapse" (Shaw and Dustin, 1997; Grakoui et al., 1999), a region of contact between a T cell and an antigen presenting cell (APC). Complementary adhesion molecules first form bonds between the T cell and APC, holding the two surfaces that constitute the contact area within about 40 nm of each other. These adhesion bonds rapidly migrate to the outer region of the contact area. The surface of the APC also displays a heterogeneous population of peptide-major histocompatibility complexes (MHC). If activation is to proceed, the homogeneous population of T cell receptors (TCR) must bind to a subpopulation of the peptides on the APC. When TCR bind to peptide-MHC, this brings the surfaces closer, within about 15 nm. The TCR-peptide-MHC bonds are found, after a short transition, in the inner region of the contact area, surrounded by a ring of adhesion bonds.

The TCR-peptide bond is weak, characterized by a rapid dissociation rate constant and a half-life in the range of 1 to 20 s (reviewed in Davis et al., 1998). Recruitment of CD4 or CD8 coreceptors on T cells that bind to MHC molecules helps stabilize the TCR-peptide-MHC bond and increase the half-life (Garcia et al., 1996), but even T cells deficient in coreceptors or with blocked coreceptors can be activated (Hampl et al., 1997). T cell activation has been triggered with APCs having fewer than 100 specific peptide-MHC on

their surfaces. Further, over a period of hours during which the immunological synapse is maintained, thousands of TCR are internalized. This observation led Valitutti et al. (1995) to propose that a single peptide-MHC can interact with many TCR, often causing the TCR to be internalized. They estimated that when there was a low density of specific peptide-MHC on the APC, a single peptide could serially engage 200 TCR, i.e., the ratio of TCR internalized to peptide-MHC on an APC was approximately 200. In different experiments, Itoh et al. (1999) observed down-regulation of 80 to 100 TCR per peptide-MHC. However, serial engagement has been challenged by San José et al. (2000), who showed that TCR that have not been engaged by peptide-MHC can be internalized in a peptide-MHC-dependent manner. This observation raises the possibility that the internalization of more TCR than peptides on the APC may be due to a bystander effect rather than serial engagement.

We present here a way to decide whether serial engagement occurs under physiological conditions that are independent of TCR internalization. Our approach is to determine how serial engagement depends on the parameters governing the T cell-APC interaction, i.e., the forward and reverse rate constants for the TCR-peptide bond, the surface densities of peptide-MHC and TCR, the radius of the contact area, and the diffusion coefficients of the TCR, peptide-MHC, and the bound complex that they form with each other. We derive analytic expressions, in terms of the fundamental parameters, for the total number of TCR bonds formed, serially, by a single peptide-MHC, before the complex diffuses out of the contact area, and for the rate of TCR bond formation per peptide-MHC. Applying these expressions to data, we obtain bounds on the number of TCR encounters per peptide-MHC, for peptides with distinct binding properties and signaling behavior.

RESULTS

Mean residence time for a particle alternating between two diffusing states

The analysis starts with a model in which a particle diffuses in a circular region of radius a, alternating between state 1, where it diffuses with diffusion coefficient D_1, and state 2, where its diffusion coefficient is D_2 (Fig. 1). The transition from state 1 to state 2 occurs at a rate λ_1 and the transition from state 2 to state 1 occurs at a rate λ_2. We calculate the mean residence time for the particle in the region, or equivalently, the mean time until the particle crosses the boundary of the region. For the case where the diffusing particle is a peptide-MHC in an immunological synapse, alternating between unbound and TCR-bound states, the calculation gives the mean time the complex remains in the contact area between the APC and the T cell.

In the Appendix, we show that the mean residence time, $\langle t_1 \rangle$, for a particle starting in state 1 at a random location within the circular region, is given by

$$\langle t_1 \rangle = \left(\frac{a^2}{8} \right) \left(\frac{\lambda_1 + \lambda_2}{\lambda_1 D_2 + \lambda_2 D_1} \right)$$

$$+ \frac{\lambda_1 D_2 (D_2 - D_1)}{(\lambda_1 D_2 + \lambda_2 D_1)^2} \left(1 - \frac{2 I_1(\alpha a)}{\alpha a I_0(\alpha a)} \right) \quad (1)$$
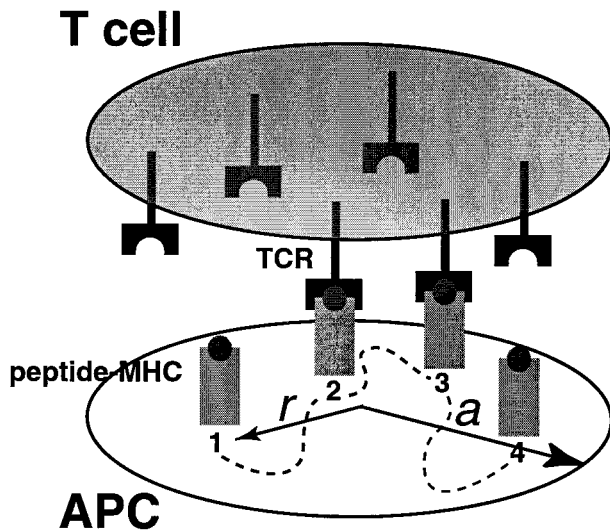

FIGURE 1 Peptide-MHC diffuse on an antigen-presenting cell (APC) and bind to T cell receptors (TCR) on a T cell, in the region of contact of the two cells. In the model used to calculate the number and rate of serial TCR encounters by a peptide-MHC, the contact area is taken to be circular, with radius a. The initial position of the complex is specified by the distance r from the center of the region. The diffusion coefficient governing movement of the peptide-MHC is D_1 when the complex is not bound to a TCR (pathway 1 to 2; then binding occurs at position 2). The diffusion coefficient is D_2 when the complex is TCR-bound (pathway 2 to 3 to 4; dissociation occurs at position 4).

where I_0 and I_1 are modified Bessel functions (Abramowitz and Stegun, 1964) and $\alpha = ((\lambda_1/D_1) + (\lambda_2/D_2))^{1/2}$.

Application to free and TCR-bound peptide-MHC

For a peptide-MHC that is either free (state 1) or bound to a TCR (state 2), the transition rates are

$$\lambda_1 = \bar{k}_{\mathrm{on}} T \quad \lambda_2 = k_{\mathrm{off}} \quad (2)$$

where $\bar{k}_{\mathrm{on}}$ is the effective two-dimensional forward rate constant (Dustin et al., 1996; Shaw and Dustin, 1997) for the binding of peptide-MHC to TCR, within the contact area between an APC and T cell, k_{off} is the reverse rate constant, and T denotes the concentration of unbound TCR in the contact area. We make the approximation that T is constant and uniform in the contact area over the period of interest. The estimates we present are for low densities of peptide-MHC, so that binding does not significantly deplete the pool of unbound TCR, nor do peptide-MHC compete among themselves for unbound TCR. Further, the estimates hold only for short times, before internalization and other processes cause extensive down-regulation of TCR or spatial redistribution of TCR and peptide-MHC in the contact area.

There are three diffusion coefficients of interest, D_{P} for the free peptide-MHC on the APC, D_{T} for the free TCR on the T cell, and D_{B} for the bound complex. If, when a bridge forms between a TCR and a peptide-MHC, there are no induced cytoskeletal interactions, then from the Einstein relation it follows that the diffusion coefficient of the bound complex is

$$D_{\mathrm{B}} = \frac{D_{\mathrm{P}} D_{\mathrm{T}}}{D_{\mathrm{P}} + D_{\mathrm{T}}} \quad (3)$$

From Eq. 3, we see that $D_{\mathrm{B}} \leq D_{\mathrm{P}}$. If the cell-cell bridge formed by a bound TCR-peptide-MHC induces cytoskeletal constraints beyond those that act separately on unbound mobile TCR and peptide-MHC, the bound complex may be essentially immobile; then $D_{\mathrm{B}} \approx 0$.

In the notation of the general model, $D_1 = D_{\mathrm{P}}$ and $D_2 = D_{\mathrm{B}}$. The expression for the mean residence time in the contact area (Eq. 1) simplifies in the two extreme cases:

$$\langle t_1 \rangle = \frac{a^2}{8 D_{\mathrm{P}}} \quad \text{if } D_{\mathrm{B}} = D_{\mathrm{P}} \quad (4)$$

$$\langle t_1 \rangle = \left(\frac{a^2}{8 D_{\mathrm{P}}} \right) \left(1 + \frac{\lambda_1}{\lambda_2} \right) = \frac{a^2}{8 D_{\mathrm{P}}} (1 + \bar{K} T) \quad \text{if } D_{\mathrm{B}} = 0 \quad (5)$$

where $\bar{K} = \bar{k}_{\mathrm{on}}/k_{\mathrm{off}}$ is the two-dimensional equilibrium constant.

Number and rate of TCR hits per peptide-MHC

We can now estimate the number and rate of encounters ("hits") between a peptide-MHC and T cell receptors in an

immunological synapse. The number of TCR hits by a single peptide-MHC, while it remains in the contact area, is approximately equal to the mean time in the contact area, $\langle t_1 \rangle$, divided by the mean cycle time, i.e., the sum of the mean time for a free peptide-MHC to bind to a TCR, $1/(\bar{k}_{on}T)$, and the mean lifetime of the resulting bond, $1/k_{off}$. Then

$$\text{hits} \approx \frac{1}{(1/(\bar{k}_{on}T)) + (1/k_{off})}\langle t_1 \rangle = \frac{k_{off}\bar{K}T}{1 + \bar{K}T}\langle t_1 \rangle \quad (6)$$

The hitting rate per peptide is the number of hits divided by the time a peptide spends in the contact area

$$\text{hits/s} \approx \frac{k_{off}\bar{K}T}{1 + \bar{K}T} \quad (7)$$

From Eq. 7, we see that the hitting rate depends only on the two-dimensional equilibrium constant and the reverse rate constant, or equivalently, on the forward and reverse rate constants. The hitting rate does not depend on the diffusion coefficients or the radius of the contact area. An alternative way to obtain the hitting rate is simply to note that there is one hit per mean cycle time.

Limiting cases

Two limits are helpful in understanding what determines the rate of serial engagement, for different peptides

$$\text{hits/s} \approx \begin{cases} k_{off} & \text{if} \quad \bar{K}T \gg 1 \\ \bar{k}_{on}T & \text{if} \quad \bar{K}T \ll 1 \end{cases} \quad (8)$$

When $\bar{K}T \gg 1$, so that a peptide-MHC complex spends a large fraction of its time bound to TCR, the dissociation rate determines the hitting rate. In this limit, increasing the TCR concentration no longer increases the hitting rate. The TCR concentration is already sufficiently high that when a peptide-MHC complex dissociates, it will immediately find and bind to a new TCR. In the other limit, when $\bar{K}T \ll 1$, the rate of hitting is determined by the rate of binding. In this case, dissociation is so rapid relative to binding that the time between TCR hits is essentially the time it takes a free peptide-MHC to bind to a TCR.

If we consider peptide-MHC complexes with similar rates of binding to TCR in an immunological synapse, but with a wide range of dissociation rate constants, the hitting rates can range from near 0 (when k_{off} is so small that $\bar{K}T \gg 1$ and the hitting rate is approximately k_{off}) up to $\bar{k}_{on}T$ (when k_{off} is so large that $\bar{K}T \ll 1$).

Potentially, the total number of TCR hits while a peptide-MHC remains in the contact area (Eq. 6) depends on all of the system parameters, through $\langle t_1 \rangle$ (Eq. 1). However, if the TCR-bound complex is immobile, then $\langle t_1 \rangle$ is given by Eq.

5 and

$$\text{hits} \approx \frac{\bar{k}_{on}Ta^2}{8D_P} \quad \text{if } D_B = 0 \quad (9)$$

Equation 9 shows that in the special case where diffusion of the bound complex is negligible, the total number of hits is independent of the reverse rate constant k_{off}. The hitting rate, however, depends on k_{off}.

Estimates from data

We can now use the general expression we derived for the hitting rate (Eq. 7) to see if, under typical experimental conditions, serial engagement occurs. The two-dimensional dissociation constant, $\bar{K}_D = 1/\bar{K} = k_{off}/\bar{k}_{on}$, has been determined to be 1×10^9 cm^{-2} ($\bar{K} = 1 \times 10^{-9}$ cm^2) for the binding of TCR to the peptide MCC88-103 complexed with mobile MHC molecules on a planar bilayer (Grakoui et al., 1999). For this peptide, $k_{off} = 0.057$ s^{-1}. For a T cell with 3×10^4 TCR per cell (Shaw and Dustin, 1997) and a surface area $S = 5 \times 10^{-6}$ cm^2 (Grakoui et al., 1999), the TCR concentration at the start of the experiment $T = 6 \times 10^9$ cm^{-2}, and $\bar{K}T = 6$. From Eq. 7, the hitting rate therefore equals 0.05 s^{-1}.

We can estimate how many hits a single peptide makes while in the contact area by multiplying this hitting rate by the average time a peptide spends in the contact area, $\langle t_1 \rangle$. The radius of the contact area is about 5×10^{-4} cm (Grakoui et al., 1999). We assume the diffusion coefficient of the MHC on an APC is typical of transmembrane proteins (reviewed by Saxton and Jacobson, 1997) and take $D_P = 3 \times 10^{-10}$ cm^2/s. For major histocompatibility antigens on various cell lines $D_P \approx 1 - 4 \times 10^{-10}$ cm^2/s (Wade et al., 1989; Edidin et al., 1991; Qui et al., 1996; Munnelly et al., 2000). From Eqs. 4 and 5 we know that $a^2/(8D_P) \leq \langle t_1 \rangle \leq (1 + \bar{K}T)a^2/(8D_P)$ and for the case being considered, 100 s $\leq \langle t_1 \rangle \leq 700$ s. (The lower bound corresponds to both the free and bound peptide diffusing at the same rate, and the upper bound corresponds to the bound complex being immobile.) Thus, we estimate that at the start of an experiment, when APC and T cell have attached and a contact area has formed, a peptide initially in the contact area will engage 5 to 35 TCR in the period of 100 to 700 s (2 to 12 min) before leaving the contact area. Over a 5-h period, as in the experiments of Valitutti et al. (1995), a peptide will enter and leave the contact area numerous times, engaging additional TCR. For the set of parameters we have considered, we therefore predict that a significant number of serial engagements occur. (From the results we have derived, we cannot estimate how many serial encounters occur over long periods of time, since T will decrease with time and become nonuniform as TCR internalization occurs, and the mean time a peptide-MHC spends in the contact area will change as complexes enter the region from the periphery.)

Effect of internalization

Internalization of TCR, and other processes that may contribute to TCR down-regulation on a time scale consistent with published data (Valitutti et al., 1995; San José et al., 2000; Niedergang et al., 1997) do not affect our estimates of the extent of serial engagement in the initial period of cell-cell contact. It is easy to apply our general model to the case where bound TCR are subject to internalization. In this case, there are two ways for a peptide-MHC to make a transition from state 2 (bound) to state 1 (free), i.e., by internalization of the bound TCR (at rate λ) or by dissociation of the bond between the TCR and the peptide-MHC (at rate k_{off}). Then $\lambda_2 = k_{off} + \lambda$ and the hitting rate is

$$\text{hits/s} \approx \frac{1}{(1/(\bar{k}_{on}T)) + (1/(k_{off} + \lambda))}$$

$$= \frac{k_{off}\bar{K}T}{1 + (\bar{K}T/(1 + (\lambda/k_{off})))} \quad (10)$$

To obtain a rough estimate of λ, in Fig. 2 we fit a simple exponential decay to the data of Valitutti et al. (1995) and obtained $\lambda \approx .028/\text{min}$ or $4.7 \times 10^{-4}/\text{s}$ when the peptide density is 25 nM and $\lambda \approx .038/\text{min}$ or $6.3 \times 10^{-4}/\text{s}$ when the peptide density is 20 μM. For most TCR-peptide-MHC, $k_{off} \geq 0.01 \text{ s}^{-1}$ (Davis et al., 1998). Then $\lambda/k_{off} \ll 1$, and
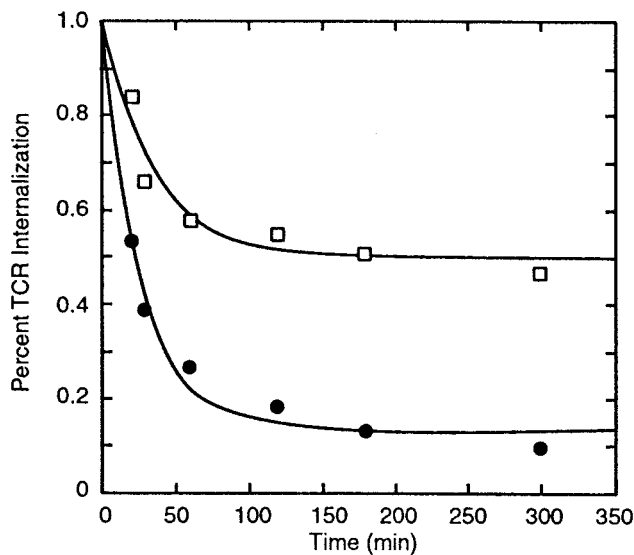

FIGURE 2 The data, from Fig. 1b of Valitutti et al. (1995), show the time course of CD3 down-regulation when T cells are conjugated with peptide-pulsed APC at peptide concentrations of 25 nM ($\square$) and 20 μM ($\bullet$), corresponding to approximately 50 and 1500 peptides per APC (see Valitutti et al., 1995, for details). The *solid lines* are separate nonlinear least squares fits to these data of the function: $\exp(-\lambda t) + f_d$, where λ is the apparent internalization rate and f_d is the fraction of TCR remaining after down-regulation is complete. The best fit values of the parameters are: $\lambda = 0.028 \pm 0.005, f_d = 0.50 \pm 0.02$ ($\square$); $\lambda = 0.038 \pm 0.003, f_d = 0.14 \pm 0.02$ ($\bullet$).

Eq. 10 indicates that internalization does not alter the previously estimated rate (Eq. 7) of serial TCR encounters by peptide-MHC in the immunological synapse.

Serial engagement of TCR by peptide agonists, weak agonists, and antagonists

To estimate the hitting rate (Eq. 7), we must know k_{off} and the two-dimensional equilibrium binding constant, $\bar{K}$. As discussed, for the one peptide (MC88-103) TCR interaction for which $\bar{K}$ has been determined (Grakoui et al., 1999), $\bar{K} = 1 \times 10^{-9} \text{ cm}^2$, $k_{off} = 0.057 \text{ s}^{-1}$ and, therefore, $\bar{k}_{on} = 5.7 \times 10^{-11} \text{ cm}^2/\text{s}$. Grakoui et al. (1999) determined from a biosensor study that the three-dimensional forward rate constant $k_{on} = 900 \text{ M}^{-1} \text{ s}^{-1}$.

If we assume that for all the peptides they studied, $\bar{k}_{on}$ is proportional to k_{on}, we can estimate hitting rates and upper and lower bounds on the number of peptide-TCR serial encounters. This is done in Table 1, where we see that serial engagement is predicted to occur for agonists, weak agonists, and antagonists.

In reaching this conclusion, we assumed that the mean time for a peptide-MHC to dissociate from a TCR is the same as that obtained from measurements where one of the reactants is in solution, such as in a BIACORE experiment. This assumption will break down if there is a significant probability that when the bond between a TCR and a peptide-MHC dissociates, the peptide-MHC will rebind to the same TCR. Such rebinding will increase the effective lifetime of the bond, i.e., reduce the value of k_{off}, and, from Eq. 7, reduce the hitting rate. Dustin (1997) investigated this rebinding question for the binding of the glycoprotein CD2 on T cells to CD58 on glass-supported planar bilayers and showed that dissociation of CD2-CD58 bonds led to the creation of new partners rather than reformation of the same pairs. Apparently, the relative diffusion between a CD2 and CD58 pair upon dissociation was such that the two molecules became well separated. Is this true as well for TCR/peptide-MHC pairs in the contact region? In the experiments of Grakoui et al. (1999), 80% of the TCR population appeared to be immobile in a fluorescence photobleaching recovery experiment. Let us estimate what the diffusion coefficient of the peptide-MHC, D_p, must be to achieve TCR/peptide-MHC separation after dissociation, in the extreme case when all TCR are immobile. Upon dissociation, in a time t the peptide-MHC diffuses a mean square distance $r^2 = 4D_p t$. Assuming re-formation of the same pair does not occur, the peptide-MHC will diffuse a mean time $\Delta t = 1/(\bar{k}_{on}T)$ before binding another TCR. (Recall that $\bar{k}_{on}$ is the effective two-dimensional forward rate constant and T is the concentration of unbound TCR in the contact area.) If the peptide-MHC diffuses a distance that is less than the average distance between TCR, then rebinding to the same TCR may become significant. For randomly distributed TCR on a surface, the mean square distance between TCR is $\langle s^2 \rangle =$

TABLE 1   Estimates of hitting rates and initial number of hits for peptide-MHC interacting with TCR

Ligand	Type	K (nM^{-1})	k_{on} (s^{-1})	k_{on} (M^{-1} s^{-1})	$\bar{k}_{on}$ (cm^2/s)	$\bar{K}$ (cm^2)	$\bar{K}T$	Hitting rate per peptide (s^{-1})	Initial hits*
				Cytochrome system					
MCC88-103	Agonist	16.6	0.057	900	5.7×10^{-11}	1.0×10^{-9}	6.0	0.05	5–36
T102S	Weak agonist	4.2	0.36	1500	9.5×10^{-11}	2.6×10^{-10}	1.6	0.22	23–59
T102G	Antagonist	0.66	5.0	3400	2.2×10^{-10}	4.3×10^{-11}	0.25	4.0	417–521
				Hemoglobin system					
Hb64-76	Agonist	83	0.064	5557	3.5×10^{-10}	5.5×10^{-9}	33	0.062	6–259
N72T	Weak agonist	101	0.14	15374	9.7×10^{-10}	7.2×10^{-9}	43	0.13	14–610

The values of K, k_{off}, and k_{on} are from Table 1 of Grakoui et al. (1999). The two-dimensional forward rate constant $\bar{k}_{on}$ for the peptide MCC88-103 was calculated from its k_{off} value and the two-dimensional dissociation constant $\bar{K}_D = 1/\bar{K} = 1 \times 10^9$ cm^{-2}, determined in Grakoui et al. (1999). For the other peptides, k_{on} was calculated by assuming that the proportionality constant between $\bar{k}_{on}$ and k_{on} was the same as for MCC99-103. $\bar{K} = \bar{k}_{on}/k_{off}$. The last three columns were calculated for a T cell having 3×10^4 TCR, a surface area $S = 5 \times 10^{-6}$ cm^2, so that $T = 6 \times 10^9$ cm^{-2}, and a circular contact area between APC and T cell of radius 5×10^{-4} cm. (By contact area we mean the region in which the TCR-peptide bonds are confined.) The hitting rate per peptide was calculated from Eq. 7. * Initial hits is the average number of hits a peptide-MHC makes before leaving the contact area, if it starts at a random position in the contact area and the TCR concentration remains uniform at concentration T while the peptide-MHC diffuses in the contact area. The two estimates are upper and lower bounds obtained by assuming either that the TCR-peptide bond is immobile or that it diffuses with the same diffusion coefficient as the unbound peptide-MHC.

$1/(\pi T)$. Thus we expect rebinding of the same pairs to be negligible and k_{off} in the contact area to be the intrinsic off rate constant when $D_p > \langle s^2 \rangle/(4\Delta t) = \bar{k}_{on}/(4\pi)$. The peptide in Table 1 with the largest forward rate constant, $\bar{k}_{on} = 9.7 \times 10^{-10}$ cm^2/s, is the weak agonist N72T. The inequality predicts that if $D_p > 8 \times 10^{-11}$ cm^2/s, re-formation of the same TCR-peptide-MHC bond will be negligible and k_{off} in the contact region will be the same as determined from solution measurements. Although the measured value, $D_P \approx 1 - 4 \times 10^{-10}$ cm^2/s (Wade et al., 1989; Edidin et al., 1991; Qui et al., 1996; Munnelly et al., 2000), is close to the predicted value for re-formation of a TCR-peptide-MHC bond, indirect evidence suggests that k_{off} is not reduced in the contact area. The off rate constant or, equivalently, the mean lifetime of the TCR-peptide MHC bond, is the dominant factor in determining whether a peptide is an agonist, weak agonist, or antagonist (Matsui et al., 1994; Lyons et al., 1996; Kersh et al., 1998). N72T is a weak agonist with an intrinsic $k_{off} = 0.14$ s^{-1}. If k_{off} was reduced by a factor of just 2 or 3 in the contact area, we would expect N72T to be a full agonist (see Table 1). Since N72T is not a full agonist, we conclude that its intrinsic k_{off} characterizes bond dissociation in the contact area as well as in solution.

DISCUSSION

We have derived an expression, Eq. 6, for the number of TCR a peptide-MHC binds, sequentially, before diffusing out of the immunological synapse, assuming the peptide-MHC starts from a random position within the contact area. From this expression we obtained the per peptide hitting rate, Eq. 7. We also obtained expressions for the shortest and longest times a peptide-MHC spends in the contact area, Eqs. 4 and 5. These times correspond to the two extremes, where the motion of the peptide-MHC is not influenced by

binding to the TCR and where the peptide-MHC becomes immobile upon forming a bond. We used these results to see if serial engagement occurs at the start of an experiment, when an APC and T cell have come into contact, and the peptide-MHC and TCR concentrations are still uniform in the contact area. We estimated, for each of a series of peptides, lower and upper bounds on the number of hits (TCR engagements) a peptide-MHC makes before it leaves the contact area (Table 1).

The estimates of serial engagement rates, per peptide-MHC, depend on the TCR concentration being approximately uniform in the contact area during the first few minutes of the experiment but are independent of how TCR are transported on the T cell surface. The predictions are the same whether active (Valitutti et al., 1995; Wülfing and Davis, 1998) or passive (diffusive) transport mechanisms dominate the movement of TCR into the immunological synapse. The estimates show that for agonists (i.e., peptides that trigger T cell responses), weak agonists, and antagonists, serial engagement occurs.

Previous arguments supporting serial engagement (Valitutti et al., 1995; Itoh et al., 1999; Lanzavecchia and Sallusto, 2000) have been based on the observation of extensive TCR down-regulation, apparently triggered by a relatively small number of peptide-MHC. If bystander effects lead to the internalization of more than one TCR per peptide-TCR encounter (San José et al., 2000; Niedergang et al., 1997), published estimates of the number of serial engagements per peptide (Valitutti et al., 1995) may be high. Nevertheless, for the parameters that characterize peptide-TCR interactions, serial engagement is expected to be robust.

If all peptide-MHC that interact with TCR, whether agonists, weak agonists or antagonists, undergo serial engagement, what role does serial engagement play in T cell

activation? The simple formation of bonds between TCR and peptide-MHC within the immunological synapse is probably not sufficient to initiate a T cell response. As with all other multisubunit immune recognition receptors (MIRR), there is considerable evidence that receptor aggregation must occur before a TCR can initiate a signaling cascade (Boniface et al., 1998; Bachmann et al., 1998; Bachmann and Ohashi, 1999). Oligomerization of TCR bound to peptide-MHC is followed rapidly by a series of TCR modifications. First, specific tyrosines residing in immunoreceptor tyrosine-based activation motifs (ITAMs) on subunits of the TCR-CD3 complex are phosphorylated by the protein tyrosine kinase (PTK) Lck. This results in recruitment of the PTK ZAP-70 from the cytosol to the phosphorylated TCR ζ chain. Zap-70 in turn becomes phosphorylated and, as long as the signaling complex remains intact, the signaling cascade proceeds (reviewed in Germain and Stefanova, 1999; Lanzavecchia et al., 1999).

The mean lifetime of the TCR-peptide-MHC bond ($1/k_{off}$) appears to be the dominant factor in determining whether a peptide is an agonist, weak agonist, or antagonist (Matsui et al., 1994; Lyons et al., 1996; Kersh et al., 1998). From Table 1 we see that there is a strong correlation between k_{off}, the hitting rate, and a peptide's ability to activate T cells. In Table 1, agonists have the smallest k_{off} values and slowest hitting rates and the antagonist has the highest k_{off} and the fastest hitting rate. However, a second mechanism, kinetic proofreading, has been invoked to explain the correlation between T cell activation and the lifetime of the TCR-peptide-MHC bond (McKeithan, 1995). The kinetic proofreading model postulates that for a particular cellular response to occur, the TCR must complete a series of modifications (e.g., phosphorylations, associations with enzymes, adaptors). If the lifetime of the TCR-peptide-MHC bond is too short, then almost always, a bound TCR will dissociate from the peptide-MHC, become disengaged from any signaling molecules it has associated with, and be dephosphorylated to its basal level before it undergoes the necessary number of modifications to achieve activation. As observed, the kinetic proofreading model predicts that the best agonist peptides will have the longest TCR-peptide-MHC bond lifetimes.

If kinetic proofreading is all that matters, then the longer the lifetime (the smaller the k_{off}) of the TCR-peptide-MHC bond, the better the peptide will be at activating T cells. If that is so, why have no agonists been reported with half-lives longer than about 30 s (Corr et al., 1994; Lyons et al., 1996; Grakoui et al., 1999; Davis et al., 1999)? Lanzavecchia et al. (1999) have argued that in addition to the lifetime of the TCR-peptide-MHC bond, of key importance in T cell activation is the number of triggered TCR. For a fixed k_{on}, as k_{off} decreases, the hitting rate (Eq. 7), decreases. Thus, what appears to place an upper limit on the lifetime of an agonist peptide is the balance between the lifetime of the TCR-peptide-MHC bond and the hitting rate.

We noted that in Table 1, the antagonist has the highest hitting rate. One way antagonism can occur is if a critical initiating kinase is limiting, as is the initiating kinase Lyn for the MIRR FcϵRI, in rat basophilic leukemia cells (Torigoe et al., 1997; Wofsy et al., 1997). As proposed by Torigoe et al. (1998), the initiating kinase can be tied up in unproductive associations with TCR that are repeatedly forming short-lived bonds with antagonist peptide-MHC. The result is that less initiating kinase is available for association with TCR that form long-lived bonds with agonist peptide-MHC. A second way the agonist may be inhibited is through the formation of mixed oligomers composed of TCR-agonist bonds and TCR-antagonist bonds. These heterogeneous oligomers will have shorter lifetimes than oligomers formed solely by TCR-agonist bonds and be less effective at signaling (Davis et al., 1998). Of course, if the lifetime of the TCR-antagonist bond becomes too short, even the initiating kinase will not have time to associate. This cannot be compensated for by raising the hitting rate and creating more oligomers, because in the limit of large k_{off}, the hitting rate becomes independent of k_{off} and approaches its maximum value, $\bar{k}_{on} T$ (Eq. 9). The balance between hitting rates and the TCR-peptide-MHC bond lifetime, i.e., between serial engagement and kinetic proofreading, plays a dominant role in determining the range of k_{off} values over which peptides are active, whether as agonists, weak agonists, or antagonists.

APPENDIX

Derivation of mean time in the contact area

For a particle diffusing in a circular region of radius a, with an initial position $r \leq a$ and initial state i ($i = 1, 2$), we define t_i as the mean time to reach the boundary of the region. The mean residence times t_1 and t_2 satisfy the partial differential equations

$$D_1 \nabla^2 t_1 - \lambda_1 t_1 + \lambda_1 t_2 + 1 = 0 \qquad (11)$$

$$D_2 \nabla^2 t_2 - \lambda_2 t_2 + \lambda_2 t_1 + 1 = 0 \qquad (12)$$

where D_1, D_2, λ_1, and λ_2 are the diffusion coefficients for the two states, and rates of transition between the states, defined previously. A heuristic derivation of Eqs. 11 and 12, based on a two-dimensional random walk, is analogous to the derivation of Eqs. 10a, b presented in Appendix A of Goldstein et al. (1984). The boundary conditions for the application we consider here are $t_i(a) = 0$ and $t_i(0)$ is finite, for $i = 1, 2$.

Multiplying Eq. 11 by λ_2 and Eq. 12 by λ_1 and adding the resulting equations gives Poisson's equation for an average t

$$D \nabla^2 t = -1 \qquad (13)$$

where

$$D = \frac{\lambda_2}{\lambda_1 + \lambda_2} D_1 + \frac{\lambda_1}{\lambda_1 + \lambda_2} D_2 \qquad (14a)$$

$$t = \frac{\lambda_2 D_1}{\lambda_1 D_2 + \lambda_2 D_1} t_1 + \frac{\lambda_1 D_2}{\lambda_1 D_2 + \lambda_2 D_1} t_2 \qquad (14b)$$

The solution to Eq. 13, subject to the boundary conditions, is $t(r) = (a^2 - r^2)/(4D)$. Using the definition of t (Eq. 14b) to eliminate t_2 in Eq. 11 gives this equation for t_1:

$$D_1 \nabla^2 t_1 - (\lambda_1 + \lambda_2 D_1/D_2)t_1 = -1$$
$$- (\lambda_1 + \lambda_2 D_1/D_2)(a^2 - r^2)/(4D) \quad (15)$$

The general solution is the following sum of the general solution to the corresponding homogeneous equation (Abramowitz and Stegun, 1964) and a particular solution to Eq. 15:

$$t_1(r) = AI_0(\alpha r) + BK_0(\alpha r)$$
$$+ (a^2 - r^2)/(4D) + \frac{(1 - D_1/D)(D_2/D)}{\lambda_1 + \lambda_2} \quad (16)$$

where I_0 and K_0 are modified Bessel functions and $\alpha = ((\lambda_1/D_1) + (\lambda_2/D_2))^{1/2}$. The function K_0 becomes infinite as r tends to 0, so to maintain a finite solution, we need $B = 0$. Applying the other boundary condition, $t_1(a) = 0$, gives

$$t_1(r) = \frac{a^2 - r^2}{4D} + \frac{(1 - D_1/D)(D_2/D)}{\lambda_1 + \lambda_2}\left(1 - \frac{I_0(\alpha r)}{I_0(\alpha a)}\right) \quad (17)$$

Averaging over r, we obtain Eq. 1.

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