Mathematical modeling of the kinetic-segregation-model without moving boundaries

(*i.e.* without integration of TCR resistance times over a growing close-contact)

The KS-model is distinct from other models that propose that the TCR needs to undergo conformation change or aggregation upon pMHC binding to induce phosphorylation. The only requirement of the KS-model is that the TCR stays accessible to kinases within close contact zones (ccz), protected from phosphatases that would otherwise terminate signaling. The pMHC ligands serve therefore only to enhance TCR trapping to increase its residence time inside the ccz (Davis et al. 1998, Davis et al. 2006). The relationship of the parameters in the KS-model can be described in simple terms. The mean time an unbound TCR reside in the ccz is: $r^2/(8D)$, where r is the radius of the ccz and D is the diffusion coefficient (Wofsy et al. 2001). The mean time it takes for a TCR to find a pMHC is: $1/(k_{on}M)$ where k_{on} is the 2D on-rate and M is the density of pMHCs. By dividing $r^2/(8D)$ with $1/(k_{on}M)$ one can calculate number of TCR engagements to pMHCs before the TCR diffuses out from the ccz (Jansson 2010). Multiplying the number of engagements with the lifetime of the TCR-pMHC complex ($1/k_{off}$), generates the following function that describes the mean residence time of TCRs within the ccz:

$$\tau = \frac{r^2}{8D}\left(1 + KM\right) , \qquad \text{(Equation 1)}$$

where K is the association constant for pMHC. This simple function actually describes the key mechanisms of the KS-model. Note that the function ignores the boundary effects of diffusion of bound TCRs and assumes a constant density of unbound pMHCs. Given that the residence time of unbound TCRs follows exponential distribution, the probability that an unbound TCR (K=0 in equation 1) stay within the ccz for longer than t_{min} seconds can be expressed as:

$$P_{unbound} = e^{-t_{\min}/\tau} = e^{-t_{\min}/(\mathrm{r}^2/8\mathrm{D})} . \qquad \text{(Equation 2)}$$

By substituting the radius (r) by the area (A) of the ccz, the equation is given by:

$$P_{unbound} = e^{-t_{\min}/(\mathrm{A}/\pi 8\mathrm{D})} . \qquad \text{(Equation 3)}$$

Equation 2 and 3 predict the probability that a TCR stay longer than t_{min} seconds for a ccz with fixed size. However, when the cells in our experiments land on the surface, the size of the ccz increases over time. To account of an increasing area over time, the area (A) can be substituted by the growth rate and time:

$$P_{unbound} = e^{-t_{\min}/(\mathrm{g}\cdot\mathrm{t}/\pi\cdot 8\cdot\mathrm{D})} , \qquad \text{(Equation 4)}$$

where g is the growth rate ($\mu m^2/s$) of the ccz and t is the time (s). Hence, once the ccz grow larger over time, the probability that an unbound TCR will stay longer than t_{min} seconds will increase. The equation predicts that the time when a cells land on the surface until the time TCR triggering is observed (time of Ca^{2+} release) will mainly be controlled by the growth rate of the ccz. To be able to describe the relationship between time to triggering (τ_{trigg}) and growth rate (g), we can reformulate equation 4 as:

$$t_{trigg} = -\frac{t_{\min}}{\log(P_{unbound})} \cdot \frac{8 \cdot D \cdot \pi}{\mathrm{g}} \ . \qquad \text{(Equation 5)}$$

By fixing $P_{unbound}$, to an arbitrary value between zero and one, we see that the time to triggering is the reciprocal of the growth rate. Hence, equation 5 predicts a reciprocal relationship between triggering time and growth rate of the ccz.

Probability of LIT triggering under "real" physiological conditions

In contrast to the previously derived equations, which are based on our experimental condition where cells simply land on a surface forming sustained contacts that continuously increase in size, real cell-to-cell contacts between T cells and APC are predicted to form smaller ccz and occur within a few minutes. T cells have been shown to scan the surface of an APC for about 1-5 minutes (Stoll et a. 2002, Miller et al. 2004). To be able to calculate the probability of ligand independent triggering (LIT) under such conditions; we need to estimate the number of trails, i.e. the number of TCR that has the chance of undergoing LIT during the cell-to-cell contact. Using the predicted physiological size of the ccz of 0.1 μm in radius, as proposed by Burroughs et al. (Burroughs et al. 2006), and a TCR expression level of 100 molecules per μm^2, results in that about 3 TCRs would on average fit in one ccz over time. By assuming that, on average, about 10 such ccz exists in a cell-to-cell contact, this means that about 30 TCRs will on average exists in the cczs. Using equation 1, the mean residence time these TCR spend in the ccz is 0.0125 seconds, given a diffusion rate of 0.1 $\mu m^2/s$. By assuming that the 30 TCRs are replaced by 30 new TCR after 0.0125 seconds, we can calculate the number of trails during a cell-to-cell contact. If a cell-to-cell contact exists for 3 minutes, about 450,000 trails would occur under these assumptions. The probability (p) that a single unbound TCR staying longer than 2 seconds (t_{min}) in the ccz, which is the assumed minimum time for triggering, can be calculated by equation 2 with a radius of 0.1 μm and a diffusion rate of 0.1 $\mu m^2/s$ ($P_{unbound} = 3.2 \times 10^{-70}$). The probability that a given number of unbound TCR trigger (stay longer than t_{min} seconds in the ccz) during the cell-to-cell contact can be calculated by the binomial distribution:

$$\Pr(\mathrm{X} = \mathrm{k}) = \binom{n}{k} p^k (1-p)^{n-k}, \qquad \text{(Equation 6)}$$

where *k* is number of successes, *n* is the number of trials (n = 450,000) and *p* is the probability of a success ($P_{unbound}$ = 3.2 x 10^{-70}). The probability that at least one TCR (k = 1 or greater) would stay longer than 2 second in the ccz is less than 10^{-60}. Hence, although the number of trails would increase by 1000-fold, the probability that at least one TCR would undergo LIT is essentially zero.

Similarity to the kinetic proofreading model

We have previously seen that, by dividing the residence time in the ccz ($r^2/(8D)$) with $1/(k_{on}M)$ one can calculate number of TCR engagements to pMHCs before the TCR diffuses out from the ccz by

$$hits = \frac{r^2/(8\mathrm{D})}{1/k_{on}M} \qquad \text{(Equation 7)}$$

Assuming an on-rate of 0.01 $\mu m^2/s$ and a pMHC density of 300 molecules per μm^2, the number of ligations a TCR makes during its time in the ccz is just 0.037. Hence, only one out of about 27 TCRs will bind a pMHC before diffusing out from the ccz. Even for a very fast on-rate, e.g. 0.1 $\mu m^2/s$, the time for a TCR to find a pMHC is still much longer than the residence time in the ccz. Thus, it is unlikely that a single TCR would bind more than one pMHC during its time in the ccz. By assuming that a TCR bind at maximum only one pMHC during its time in the ccz, equation 1 can be simplified to describe the time in the ccz for a TCR that bind only one pMHC during its time in the ccz:

$$\tau = \frac{r^2}{8D} + \frac{1}{k_{off}} \qquad \text{(Equation 8)}$$

Under the given conditions, the KS-model is very similar the Kinetic proofreading model (McKeithan, 1995), which explains how the T cells discriminate between self and non-self-peptides based on the life-time of TCR/pMHC complex ($1/k_{off}$). The KS-model differs from the kinetic proofreading models in that the KS-model predicts that (1) for a unphysiological big ccz, it predicts that LIT can occur (2) the ligation to agonistic pMHC induce triggering only due to increased residence time of the TCR inside the ccz (3), in theory, increasing the density of pMHC or the on-rate will increase the residence time and thus the likelihood of triggering due to rebinding effects.

Probability of triggering in the presence of pMHC

The previous derived equations (eq 2-4) can be used to predict the residence time and probability that unbound TCRs would stay longer than t_{min} second inside the ccz. These models results in single-exponential distributions of the residence time. However, allowing that the a TCR can bind a pMHC (K>0 in eq. 1) during its time inside the ccz will give rise to a biphasic distribution since two compartments exists: (1) one compartment holding unbound TCRs that are free to diffuse and (2) a compartment with bound TCRs that cannot diffuse outside the ccz. We model such compartment models with two simple differential equations:

$$\frac{dU}{dt} = -k_{on} \cdot U \cdot (\mathrm{M}_{pMHC} - \mathrm{U}) + k_{off} B - (1/\tau) \cdot U \qquad \text{(Equation 9)}$$

$$\frac{dB}{dt} = k_{on} \cdot U \cdot (\mathrm{M}_{pMHC} - \mathrm{U}) - k_{off} B \qquad \text{(Equation 10)}$$

where U is the unbound TCR and B is the bound TCRs. Unbound TCRs can either bind pMHC (M_{pMHC}) at rate k_{on}, or diffuse out from the ccz at rate 1/τ, where τ is the mean residence time. Bound TCRs will eventually dissociate at rate k_{off}. If the TCR bind to pMHC with strong affinity, these set of equations will give rise to a fast exponential phase including TCRs that do not bind to pMHC and a slow exponential phase including TCR that bind pMHC. The equations were solved with the initial TCR density of 100 molecules/μm2 (U(0) = 100) and no bound TCRs (B(0)=0), using MATLAB R2014b with the solver ode15s solver (MathWorks, Inc.). The fraction of TCRs inside the ccz at time t_{min} was calculated by dividing the number of TCRs still inside the ccz at t_{min} by the initial amount of TCRs.

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