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*J Immunol* 2007; 178:5505-5512; doi: 10.4049/jimmunol.178.9.5505

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Characterizing T Cell Movement within Lymph Nodes in the Absence of Antigen

Catherine Beauchemin, Narendra M. Dixit, and Alan S. Perelson

The recent application of two-photon microscopy to the visualization of T cell movement has presented trajectories of individual T cells within lymphoid organs both in the presence and in the absence of Ag-loaded dendritic cells. Remarkably, T cells largely move along conduits of the fibroblastic reticular cell network, and they appear to execute random walks in lymphoid organs rather than chemotaxis. In this study, we analyze experimental trajectories of T cells using computer simulations of idealized random walks. Comparisons of simulations with experimental data provide estimates of key parameters that characterize T cell movement in vivo. For example, we find that the distance moved before turning is about twice the distance between intersections in the fibroblastic reticular cell network, suggesting that at an intersection a T cell will turn onto a new fiber ~50% of the time. Although the calibrated model appears to offer an accurate representation of T cell movement, it has also uncovered inconsistencies across different experimental data sets. The Journal of Immunology, 2007, 178: 5505–5512.

Describing T Cell Movement

Experimental data of the mean $2d$ T cell displacement vs $\sqrt{t}$ time presented in Fig. 1 appears to display two distinct regimes. At small times, the displacement appears to have a quadratic dependence on $\sqrt{t}$ time (i.e., linear dependence on time), which resembles what one would expect for a particle moving in a straight line at a fixed speed. In contrast, for larger times, the displacement appears to depend linearly on the square root of time, suggesting that the cells are diffusing. For times smaller than the time scale of a single step of a random walk, a particle performing random walk will move in a straight line as it takes that step, yielding a quadratic shape at small times. For larger times, however, the consecutive steps taken by the particle in random directions yield a linear shape. This is illustrated in Fig. 2, and can be seen in Fig. 1 where we present data obtained from the works of Mempel et al. (5) and Miller et al. (6–8).

Although the manner in which T cells crawl is not fully understood, many characteristics are thought to be similar to the crawling of amebas of the lower eukaryote Dictyostelium discoideum (16). Migration is initiated when the T cell adopts a polarized (elongated) shape. This is followed by the formation of a lamellopodium at the leading edge and a uropod at the trailing edge (Ref. 17 and see Fig. 1 in Ref. 18). To move, the T cell anchors its uropod to the crawling surface, shifts all of its cytoplasm to the front, retracts the uropod, and starts another contraction-retraction cycle (see Fig. 1 in Ref. 19). Rather than moving in a fully unrestricted manner, Bajénoff et al. (1) recently showed that T cells traffic within lymph nodes by crawling along the network formed by the interconnected FRCs found in the paracortex region of lymph nodes.
In our simulations, we adopt a minimalist description of T cell motion. We let a T cell perform an unhindered random walk (diffuse) with a mean free time \( t_{\text{free}} \) during which the cell crawls in a straight line at a fixed speed \( v_{\text{free}} \). At the end of each free run, we allow for a pause time, \( t_{\text{pause}} \), during which the T cell is immobile as it reorganizes the cellular machinery, allowing it to turn. The cell then picks a new random direction, undergoes another free run, and so on. This scheme of movement is somewhat similar to the run-and-tumble motion of certain bacteria such as *Escherichia coli* (20), except that T cells reorient their lamellipodium, rather than tumble, between each free run. Averaging over the movement of many cells then allows us to determine the mean displacement of cells over time as a function of the parameters \( t_{\text{free}}, v_{\text{free}} \) and \( t_{\text{pause}} \). Comparison with experiments allows us to identify the key parameters characterizing this random walk.

**Materials and Methods**

**Experimental data**

The experimental data used to calibrate our model was taken from four different publications (5–8). These papers were chosen because they presented the mean displacement of T cells as a function of the square root of time from Miller et al. (8) (open circle), Miller et al. (7) (filled square), Miller et al. (6) (Δ), and from calculations based on individual T cell tracks used in Mempel et al. (5) (gray diamonds). SDs were reported only in Miller et al. (7, 8) and were computed from the tracks for Mempel et al. (5). Only a subset of data points is plotted for clarity.

**Theoretical expression for the mean displacement**

The \( \Gamma(1/2 + d/2) \Gamma(1/2) \) term in the formulas of Fig. 2 results from the fact that Miller et al. (6–8) and Mempel et al. (5) chose to present the mean displacement, \( \langle |\mathbf{r}| \rangle \), rather than the root mean squared displacement, \( \sqrt{\langle r^2 \rangle} \), as a function of \( \sqrt{t} \), as their y-axis. Let \( \mathbf{r} \) be the displacement of a particle at time \( t \) given that it started at the origin at time 0. If the particle is undergoing a random walk in \( d \) dimensions with diffusion coefficient \( D \), then its mean squared displacement as a function of time is given by

\[
\langle r^2 \rangle = 2 D t, \tag{1}
\]

for \( t \) much larger than the time scale of a single time step (20). This equation is also valid for particles moving in \( d \) dimensions the motion of which has been projected to \( \delta < d \) dimensions. In such a case, the mean squared displacement projected to \( \delta \) dimensions is \( \langle r^2 \rangle_\delta = 2 D t \), provided that motion in the \( x, y, z \) directions are statistically independent. Thus, we have

\[
\langle r^2 \rangle_\delta = \langle x^2 \rangle + \langle y^2 \rangle = 4 D t, \tag{2}
\]

An expression analogous to the one presented in Equation 1 for \( \langle r^2 \rangle \) can be derived for \( \langle |\mathbf{r}| \rangle \). At time \( t \), the displacement in \( d \) dimensions of a particle that started at the origin at time 0 is given by

\[
\langle |\mathbf{r}| \rangle = \sqrt{\langle r^2 \rangle} = \sqrt{2 D t}. \tag{3}
\]

Let us define

\[
\alpha_i = \frac{x_i}{\sqrt{2 D t}} \tag{4}
\]

so that \( x_i = \sqrt{2 D t} \alpha_i \) and

\[
\langle x_i^2 \rangle = 2 D t \langle \alpha_i^2 \rangle. \tag{5}
\]

Because \( x_i \), the displacement of a diffusing particle in direction \( i \), is a Gaussian random variable of mean \( \langle x_i \rangle = 0 \) and variance \( \langle x_i^2 \rangle = 2 D t \), it follows that \( \alpha_i \) is a Gaussian random variable of mean 0 and variance 1. The mean displacement in \( d \) dimensions at time \( t \) would then be given by

\[
\langle |\mathbf{r}| \rangle = \sqrt{2 D t} \left( \sum_{i=1}^{d} \alpha_i^2 \right) = \sqrt{2 D t} \langle \sqrt{\delta} \rangle, \tag{6}
\]

where \( \beta = \sum_{i=1}^{d} \alpha_i^2 \). The sum of the squares of \( d \) Gaussian random variables of mean \( 0 \) and variance \( 1 \) is a \( \chi^2 \)-distributed random variable with \( d \) degrees of freedom (E. W. Weinstein, *Chi-Squared Distribution*; http://mathworld.wolfram.com/Chi-SquaredDistribution.html). The \( 2\text{nd} \) moment of a \( \chi^2 \)-distributed random variable \( \beta \) with \( d \) degrees of freedom is given by (E. W. Weinstein; http://mathworld.wolfram.com/Chi-SquaredDistribution.html).

\[
\langle \sqrt{\beta} \rangle = \frac{\Gamma \left( \frac{d}{2} + \frac{1}{2} \right)}{\Gamma \left( \frac{d}{2} \right)}. \tag{7}
\]

Substituting this back into Equation 6, we obtain

\[
\langle |\mathbf{r}| \rangle = \sqrt{2 D t} \frac{\Gamma \left( \frac{d}{2} + \frac{1}{2} \right)}{\Gamma \left( \frac{d}{2} \right)}. \tag{8}
\]
FIGURE 3. The angles of the velocity vector, $\vec{v}$, are defined such that $\theta$ is the angle measured southward from the positive $z$-axis, and $\phi$ is the angle measured eastward from the positive $x$-axis. Notice how the area elements $\sin \theta \, d\theta \, d\phi$ (dark gray) yield different areas for different values of $\theta$.

Simulation of T cell movement

Two-photon microscopy experiments report either two-dimensional (2d) (6–8) or three-dimensional (3d) (5) coordinates of T cells in lymphoid organs as a function of time. Because the experimental resolution in 3d is lower than in 2d, 3d results can be misleading. Because 2d data provide an adequate representation for the mean displacement, we simulate the 3d motion of T cells projected onto 2d by using the standard relationship between cartesian and spherical coordinates. The algorithm we use is described below.

Let $(x_t, y_t)$ be the 2d coordinates of a T cell at time $t$. At time $t + \Delta t$, the position of the particle is given by

$$
x_{t+\Delta t} = x_t + \Delta t \cdot [\vec{v} \sin \theta \cos \phi]$$

$$
y_{t+\Delta t} = y_t + \Delta t \cdot [\vec{v} \sin \theta \cos \phi]
$$

where $[\vec{v}]$ is the magnitude of the 3d velocity of the T cell, and the angles $\theta$ and $\phi$ are as defined in Fig. 3. Thus $\vec{v} \sin \theta \cos \phi$ is the $x$-component of the T cell velocity used in Equation 9 above and $\vec{v} \sin \theta \sin \phi$ is the corresponding $y$-component used in Equation 10. Equations 9 and 10 thus allow us to simulate the 2d projection of the 3d movement of T cells without explicitly considering the $z$-component of motion.

To mimic the successive periods of running and pausing, we set $\vec{v}$ to $\vec{v}_{\text{free}}$ for a length of time $t_{\text{pause}}$, then set it to zero for a length of time $t_{\text{pause}}$, and so on. At the beginning of each free run, i.e., when $\vec{v}$ is switched from zero to $\vec{v}_{\text{free}}$, new values for $\theta$ and $\phi$ are picked at random. In order to distribute the chosen directions of $\vec{v}$ uniformly over the sphere, we choose a random $\phi$ uniformly distributed in $[0, 2\pi]$, and choose a random $\theta$ distributed in $[0, \pi)$ according to the probability density $P = \frac{1}{\pi} \sin \theta$. In this way, there is a constant density per unit area element $\sin \theta \, d\theta \, d\phi$ (Fig. 3). If $\theta$ were to be chosen evenly in $[0, \pi)$, the resulting set of velocity vectors would be more dense toward the poles.

Each T cell run lasts 20 min, and we chose a step size $\Delta t = 1$ s. To prevent artifacts, which would result from the synchronization of pause times across T cell runs, the time for the first pause in each T cell run is chosen at random, uniformly distributed in $[-t_{\text{pause}} + \Delta t, t_{\text{free}}]$ (Fig. 4).

When this value is negative, the T cell is started in a paused mode at time $t = 0$, and the magnitude of the negative number indicates for how long before $t = 0$ the cell has been in that state.

The 2d displacement of the T cell from its initial position $(x_0, y_0) = (0, 0)$ is computed using

$$r_t = \sqrt{x_t^2 + y_t^2},$$

where $r_t$ is the magnitude of the 2d displacement of the T cell from its initial position ($x_0, y_0$).

FIGURE 4. In each T cell run, the time for the first pause, $t_p$, which in turns sets the time of the first free run, $t_f$, is chosen at random uniformly over all possible choices, namely $[-t_{\text{pause}} + \Delta t, t_{\text{free}}]$. This is illustrated here for the case of $t_{\text{pause}} = 3$ time steps (the time interval during which a cell is paused) and $t_{\text{free}} = 5$ time steps (the time interval during which a cell in moving at constant speed $v_{\text{free}}$), where the duration of a time step is $\Delta t$.

FIGURE 5. The effect of varying a single parameter at a time, while maintaining the other two parameters fixed, on the shape of the 2d mean displacement curve. When fixed, the parameters are set to $t_{\text{pause}} = 0.5$ min, $t_{\text{free}} = 2$ min, and $v_{\text{free}} = 18.8 \mu m$/min. Then, $t_{\text{free}}$ is varied by increments of 0.5 min over the range [0.5, 20.0] min, $v_{\text{free}}$ is varied by increments of 1.0, and $t_{\text{pause}}$ is varied by increments of 0.00367 min/\mu m over the range [0.02, 0.2] min/\mu m, and $t_{\text{pause}}$ is varied by increments of 0.25 min over the range [0.0, 3.5] min. The simulation results were produced by averaging the individual displacement of $10^6$ T cells for each triplet $(t_{\text{pause}}, t_{\text{free}}, v_{\text{free}})$. 

\[\text{Equation 9}\]

\[\text{Equation 10}\]
for $t$ from 0 to 20 min. We repeat the above procedure for $n = 10^6$ T cells and obtain the average displacement at time $t$,

$$\langle r \rangle = \frac{1}{N} \sum_{i=1}^{N} \sqrt{x_{i,t}^2 + y_{i,t}^2},$$  

(12)

where $(x_{i,t}, y_{i,t})$ is the position of the $i$th T cell at time $t$.

Fig. 5 illustrates how each of the parameters $t_{\text{pause}}, t_{\text{free}},$ and $v_{\text{free}}$ affects the 2d mean displacement as a function of the square root of time. The most important effect of increasing $t_{\text{free}}$ is to increase the slope of the curve at larger times, whereas that of increasing $v_{\text{free}}$ is to generate a more pronounced curvature at smaller times. The effect of increasing $t_{\text{pause}}$ is more subtle but is mostly a horizontal translation toward larger times of the time of transition between the quadratic and linear regimes of the 2d mean displacement.

To compare the T cell velocities in our model, $v_{\text{free}}$, with those measured experimentally, namely the 2d velocities, $v_{\text{exp}}$, the parameter $v_{\text{free}}$ must be converted using

$$v_{\text{exp}} = v_{\text{free}} \times \left( \frac{\pi}{4} \times \frac{t_{\text{free}}}{t_{\text{free}} + t_{\text{pause}}} \right).$$  

(13)

The term $\pi/4$ is necessary to convert the 3d velocity of our simulation to its 2d equivalent for comparison with the velocities measured by Mempelet al. (5) and Miller et al. (6–8). It comes from the fact that $v_{2d} = v_{3d} \cdot \sin \theta$, where $\theta$ is as illustrated in Fig. 3. On average,

$$\langle \sin \theta \rangle = \frac{1}{\pi} \int_0^{\pi} P(\theta) \sin \theta \, d\theta = \frac{1}{\pi} \int_0^{\pi} \sin^2 \theta / 2 \, d\theta = \frac{\pi}{4}.$$  

(14)

The experimental instantaneous velocity, $v_{\text{exp}}$, is computed from the distance traveled by a T cell in the xy-plane over a short time interval ($\sim 10$ s) (5–8). Over this time interval, some T cells are paused, some T cells are moving, and some will experience both states sequentially. The term $t_{\text{free}}/ (t_{\text{free}} + t_{\text{pause}})$ accounts for this fact and converts the velocity from our simulation to the T cell velocity that would be measured experimentally.

In light of recent experiments which showed that T cells move in lymph nodes by crawling along the FRC network (1), it is of interest to compare the persistence length or mean free path of the free runs of the T cells in our model to the mean distance between intersections in the FRC network. This distance is reported to range between 5 and 37 $\mu$m, with an average of $17 \pm 7 \mu$m for the six lymph nodes studied in Ref. 1. For this purpose, we introduce the variable $d_{\text{free}}$, which is simply

$$d_{\text{free}} = t_{\text{free}} \times v_{\text{free}}.$$  

(15)

### Results

**Comparison with experiments**

For each value of the parameter triplet ($t_{\text{pause}}, t_{\text{free}}, v_{\text{free}}$), the individual trajectories of $10^6$ T cells were simulated, varying only the time of the first pause and the seed of the random number generators, and the average of their 2d displacements was computed. This was repeated as parameter values were varied over the ranges $t_{\text{pause}} = [0, 3.5]$ min, $t_{\text{free}} = [0.5, 20]$ min, and $v_{\text{free}} = [5, 50]$ $\mu$m/min. Then, for each triplet value, we computed the sum of the squared residuals (SSR) between the simulation results and an experimental data set composed of all the experimental data from the four publications (5–8). The SSR were then used to rank triplet values from best to worst fit (Table I).

From Table I, we see that the triplet value of ($t_{\text{pause}} = 0.5$ min, $t_{\text{free}} = 2$ min, $v_{\text{free}} = 18.8 \mu$m/min) minimizes the SSR between the simulation results and the set composed of the combined experimental data. If, for simplicity, one only considers fits for which there is no pause time between free runs ($t_{\text{pause}} = 0$), the doublet value of ($t_{\text{free}} = 2$ min, $v_{\text{free}} = 16.6 \mu$m/min), i.e., the 6th best-fitting triplet, minimizes the SSR between the simulation results and the data of the combined sets. Also, the experimental velocity, $v_{\text{exp}}$, obtained from the best-fitting triplet values compare well with the reported experimental T cell velocity of $\sim 11 \mu$m/min (5–8).

Table I also shows the mean free path length, $d_{\text{free}}$, for each triplet. This distance for the best-fitting triplets is about twice the distance between intersections in the FRC network, which average $17 \pm 7 \mu$m (1). This suggests that at an intersection, $\sim 50\%$ of the time a T cell will turn onto a new fiber and $\sim 50\%$ of the time the T cell will continue along its original fiber. To see this, assume that the distance between intersections in the FRC network is $L$, and that a cell has probability $p$ of continuing along the same fiber at an intersection. A T cell has a probability $(1 - p)$ of going straight for only a distance $L$ and then turning at the first intersection, a probability $p(1 - p)$ to go straight for distance $2L$ by going straight through the first intersection and then turning at the next, a probability $p^2(1 - p)$ to go straight for a distance $3L$ by going straight through the first two intersections and turning at the third, etc. Thus, we can express the average distance traveled by a T cell without branching as

$$d_{\text{free}} = L(1-p) + 2LP(1-p) + 3LP^2(1-p) + \cdots =$$

$$= L(1-p) \sum_{N=1}^{\infty} Np^{N-1} =$$

$$= \frac{L}{(1-p)} \text{ for } |p| < 1.$$  

(16)

**FIGURE 6.** The combined experimental data from Mempelet al. (5) and Miller et al. (6–8) (●) along with the best-fitting triplet ($t_{\text{pause}} = 0.5$ min, $t_{\text{free}} = 2$ min, $v_{\text{free}} = 18.8 \mu$m/min) (—I—), and the best-fitting doublet or 6th best-fitting triplet ($t_{\text{pause}} = 0$, $t_{\text{free}} = 2$ min, $v_{\text{free}} = 16.6 \mu$m/min) (—). The simulation results were produced by averaging the individual displacement of $10^6$ T cells for each triplet ($t_{\text{pause}}, t_{\text{free}}, v_{\text{free}}$).
such that for $p = 1/2$, the average distance traveled without branching is $2L$, or double the mean distance between intersections.

In the end, there is not much difference between the set of combined experimental data and the simulations obtained with the best-fitting doublet or triplet (Fig. 6). Thus, given the limited amount of available experimental data, it is not possible to narrow the range of acceptable values any further or to infer the length or even the existence of a pause time between successive free runs.

The best-fitting triplet and doublet found here should not be regarded as the only solution, i.e., the parameter values that best characterize the motion of T cells in vivo in the absence of Ag. The sixth best-fitting triplet ($t_{\text{pause}} = 0$, $t_{\text{free}} = 2$ min, $v_{\text{free}} = 16.6$ μm/min), for example, has different parameter values from that of the best-fitting triplet but does not have a significantly worse SSR (3502 μm$^2$ vs 3387 μm$^2$, a 3% difference). To help in understanding where the best-fitting triplets are located with respect to one another in the parameter space of the model, Fig. 7 presents contour plots of the SSR across the parameter space of the triplets for the set of combined experimental data.

From Fig. 7, one can see that the best-fitting triplets appear to occupy a small volume in the parameter space, represented by the white areas on the graphs. Larger quantities of more precise experimental data would be necessary to reduce these areas and constrain the uncertainty on the parameters of the model.

**Experimental discrepancies**

In the previous section, a fit to a set composed of the combined experimental data from four different publications (5–8) was presented. Inspection of Fig. 1, however, reveals discrepancies between the various data sets which use different experimental procedures. For example, data from Miller et al. (8), which tracked both CD4$^+$ and CD8$^+$ T cells in mouse explanted cervical and inguinal lymph nodes, is not as curved as the other data at small times, and data from Miller...
et al. (6), which tracked CD4\(^+\) T cells in explanted cervical and inguinal lymph nodes of asphyxiated mice, appears to continue to exhibit a quadratic dependence of the mean displacement on the square root of time when the other data have entered the linear regime. To quantitatively explore these discrepancies, we computed the SSR between the simulation results and each experimental set individually over the full parameter space of the triplets explored above (Table II). For comparison purposes, Table II also presents how the best-fitting doublet and triplet found for the combined data sets fit each experimental data set individually. Fig. 8 presents the best-fitting triplet for each set along with the best-fitting doublet and triplet for the combined set against each experimental set.

The best-fitting triplets for the data of Miller et al. (8) differ from the best-fitting triplets of the combined sets in their smaller \(t_{\text{free}}\) and larger \(v_{\text{free}}\) values. This discrepancy is illustrated in Fig. 8 where the 2\(d\) mean displacement for the data of Miller et al. (8) has a more pronounced curve at smaller times and a less pronounced slope at larger times when compared with the combined set. This suggests that according to our model, the cells in the work of Miller et al. (8) are taking shorter steps, changing direction more often, and moving faster during steps. This is echoed by the smaller mean free paths, \(d_{\text{free}}\), of the five best-fitting triplets which are in the range of 21–26 \(\mu\text{m}\).

The best-fitting triplets for Miller et al. (7) differ most notably from the best-fitting triplets of the combined sets in their smaller \(t_{\text{free}}\) and larger \(v_{\text{free}}\) values. The discrepancy is illustrated in Fig. 8 where the 2\(d\) mean displacement for Miller et al. (7) appears to plateau toward the end of the experiment (\(\sim 6.25\) min), whereas that from the latter two is noisier, and thus harder to fit. In addition, it is important to consider that the experimental procedures differed among the four experiments. Miller et al. (8) considered CD3\(^+\) T cells (both CD4\(^+\) and CD8\(^+\) T cells) and conducted the experiments in mouse explanted cervical and inguinal lymph nodes. In the data of Miller et al. (7), only CD4\(^+\) T cells were considered, and the experiment was conducted in the inguinal lymph node of anesthetized mice. In Miller et al. (6), CD4\(^+\) T cells were considered, but the mice were killed by CO\(_2\) asphyxiation, and cervical or inguinal lymph nodes were explanted and maintained at 36\(^\circ\)C under superfused medium bubbled with 95% O\(_2\) and 5% CO\(_2\). Finally, according to Mempel et al. (5), CD8\(^+\) T cells were considered, and the experiments were conducted in the popliteal lymph nodes of anesthetized mice.

These differences in the experimental procedures could possibly account for the differences seen between the four data sets. Explanted lymph nodes are maintained at 36\(^\circ\)C under superfused medium bubbled with 95% O\(_2\) and 5% CO\(_2\); to take account of the fact that the lymphoid environment is thought to operate at a relatively low oxygen tension (2). But despite these precautions, it remains

### Table II. Best triplets for each experimental data set

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<tr>
<th>Rank(^a)</th>
<th>(t_{\text{pause}}) (min)</th>
<th>(t_{\text{free}}) (min)</th>
<th>(v_{\text{free}}) ((\mu\text{m}/\text{min}))</th>
<th>(v_{\text{exp}}) (a) ((\mu\text{m}/\text{min}))</th>
<th>(d_{\text{free}}) ((\mu\text{m}))</th>
<th>SSR ((\mu\text{m}^2/\text{min}))</th>
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\(^a\) The parameter \(t_{\text{pause}}\) was varied from 0 to 3.5 min, \(t_{\text{free}}\) from 0.5 to 20 min, and \(v_{\text{free}}\) from 5 to 50 \(\mu\text{m}/\text{min}\). The triplets \((t_{\text{pause}}, t_{\text{free}}, v_{\text{free}})\) have been sorted in order of increasing SSRs between the data and the simulation results. The simulation results were produced by averaging the individual displacement of 10\(^6\) T cells for each parameter triplet. The fit to each individual data set of the best-fitting doublet and triplet for the combined data sets (see Table I) is also presented.

\(^b\) Computed using Equation 15.
that explanted lymph nodes are deprived of their normal blood and lymphatic circulations, and this might have an effect on the experimental results obtained (2, 3). Additionally, the four experiments were conducted using a variety of lymph nodes (e.g., popliteal, cervical, inguinal). Differences in the underlying structures of different lymph nodes such as the density of dendritic cells (4), or more importantly the differences in the structure of the FRC network along which the T cells crawl (1), which could differ between lymph nodes in different anatomical locations or depend on the genetic background, sex, or age of the experimental animal, could explain the differences seen in the motility patterns among the four experiments.

Overall, the experimental data do not differ too significantly from the fit to the combined data set, except for Miller et al. (6), and the model does appear to provide a fair description of T cell movements in lymph nodes.

**Discussion**

From the data obtained by two-photon microscopy, the motion of T cells appears to be consistent with the cells performing a random walk for displacements over long times and following straight trajectories over short periods of times. Consequently, we proposed a simple model for the motion of T cells in lymph nodes in the absence of Ag in which T cells move in a straight line at fixed velocity $v_{free}$ for a time $t_{free}$, pause for a time $t_{pause}$ to reposition their lamellipods and uropod as they randomly pick a new direction to move in, and so on. We have found that this simple model appears to give results that agree best with experimental results for $t_{pause} = 0.5$ min, $t_{free} = 2$ min, and $v_{free} = 18.8 \mu$m/min.

If, for simplicity, one only considers fits to the experimental data for which there is no pause time between free runs ($t_{pause} = 0$), picking $t_{free} = 2$ min, and $v_{free} = 16.6 \mu$m/min yields best agreement between the simulation results and the data. Overall, the addition of a pause time to the model does not significantly improve the agreement between the experimental data and the simulation results and as such is not absolutely necessary.

It is important that the pause time, which we have added to account for the physiological time necessary for a T cell to turn, should not alternatively be interpreted as the time for contact between a T cell and a dendritic cell. The pause time for our best fit was $t_{pause} = 30$ s, whereas dendritic cell-T cell contacts, in the absence of Ag, are consistently reported to last $\sim 3$ min (5, 6, 21).

It is also important to place our model in the context of the results of Bajenoff et al. (1) that showed T cell traffic within lymph nodes by crawling along a network of "roads" formed by interconnected FRCs. We compared the mean free paths, $d_{free} = t_{free} \times v_{free}$, computed from the best-fitting triplets to the mean distance between intersections in the FRC network. The mean free paths of 38 $\mu$m found for the best-fitting triplet and 33 $\mu$m found for the best-fitting doublet are about twice the distance between intersections in the FRC network, which averages 17 $\pm$ 7 $\mu$m (1). This suggests that at an intersection, $\sim$50% of the time a T cell will turn onto a new fiber and $\sim$50% of the time the T cell will continue along its original fiber. This is an interesting prediction, which should be easily verifiable.

In our model, the angles $\phi$ and $\theta$ are picked randomly at the beginning of each free run for each T cell. In contrast, cells traveling along the FRC strands would, at each intersection, have a choice between a restricted set of angles corresponding to the orientation of the strands available at that intersection. There is no information available regarding the angles available to the T cells at FRC strand intersections. One could, however, imagine that by picking angles at random, we are in fact picking the topology of the network onto which the T cells are crawling. The fact that subsequent T cells would pick different angles would simply mean that they have chosen a different path within the network. As more information about the topology of the FRC network becomes available, it will be possible to refine the choice of angles in our model to account for the new information.

Our model was created to be simple and efficient so as to support large-scale simulations of whole lymph nodes. For this reason, a number of simplifications were made. For instance, a fixed velocity of $v_{free}$ is assigned to all of the $10^6$ T cells simulated. In reality, the population of T cells exhibit a range of velocities with distributions as shown in Fig. 2C in Ref. 8 and Fig. 3D in Ref. 7. We have modified our simulations to explore the effect of having variable velocities across the T cell population. In the modified simulations, each of the $10^6$ T cells was assigned a different velocity for the duration of the run. The velocities assigned to each T cell...
were picked randomly from a Gaussian distribution of mean $v_{\text{free}}$ with a SD of 5 μm/min. The deviation was picked so as to resemble the distributions shown in Fig. 2C in the 2002 work of Miller et al. (8) and Fig. 3D in the 2003 work of Miller et al. (7). We found that sample simulations where T cell velocities were picked randomly from this Gaussian distribution yielded mean displacement vs time curves that were indistinguishable from those produced by the model described in the paper where all T cells were assigned the same velocity.

Although our model does not explicitly consider the FRC network, simplifies the range of velocities exhibited by T cells to a single fixed velocity, and allows a random angle to be picked before each free run rather than restrict angles according, for example, to the physiology of the FRC network, it still agrees well with the experimental data for the mean displacement and mean velocity of the T cells (5–8). This is advantageous for spatial mathematical modelers because this makes modeling T cell movement simple and efficient.

These results do not exclude the possibility that chemokine attractants may direct the motion of T cells. The possibility of a chemokine gradient guiding the movement of T cells has been considered by A. K. Chakraborty (personal communication), who showed that one can observe random walk behavior in the presence of chemokine gradients as long as their effect was sufficiently short-ranged.

A more detailed model for lymphocyte movement has been proposed by Meyer-Hermann and Maini (22) and compared against B and T cell movement data from Miller et al. (8). Their model is a modified 2d cellular automaton-type Potts model (23, 24), where a cell is represented by a collection of subunits that are the lattice sites occupied by the cell volume. Like ours, their model also suggests a run-and-pause dynamics but implements it in more detail, integrating intracellular dynamics in a generic way. The subunits that make up a cell are moved individually in the direction chosen for active movement under constraints to maintain constant cell volume. After a period of movement, the cell undergoes reshaping and the subunits are moved so as to return the cell shape to a sphere. Interestingly, like ours, the mean displacement vs time produced by their model also disagrees with that of Miller et al. (8) at early times. In addition to the mean displacement, this model also yields a distribution of lymphocytes velocities that compares well with the spread seen in vivo.

Another detailed model for the movement of T cells and dendritic cells in lymph nodes was proposed by Beltman et al. (25). They use it to investigate the possibility that T cells and dendritic cells owe the characteristics of their motility patterns (i.e., run-and-pause random walk) to the anatomical structure of lymph nodes. Like the Meyer-Hermann and Maini model, theirs is a Potts model, where each cell is represented by a collection of neighboring sites, but it is a 3d rather than a 2d model. Using this model, the authors demonstrate that the run-and-pause random walk nature of T cell and dendritic cell motility need not be an intrinsic characteristic of cell motility. Rather, these characteristics emerge as a consequence of the anatomical barriers encountered by the cells which give rise to velocity fluctuations and mean displacements that are in good agreement with experimental data.

Although these models better describe lymphocyte movement by integrating more details, they are more computer intensive than ours for equivalent mean displacement results and may not lend themselves well to large scale simulations of, for example, whole lymph nodes.

In conclusion, we have proposed a simple description of T cell movement that matches experimental data and that can be implemented in a simple and computationally efficient manner.

Acknowledgments

We thank Dr. Thorsten R. Mempel for providing the T cell trajectories used in Ref. 5 and Joost Beltman for helpful discussions. We also thank one of the referees for suggesting that we calculate $\langle d_{\text{free}} \rangle$ and compare it with the results in Ref. 1.

Disclosures

The authors have no financial conflict of interest.

References