T cell movement in diverse tissues

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Function of T cells

• T cells are crucial to clear infection from multiple tissues.
  • Naïve T cells move within the paracortex of the lymph node
  • In tissue sites, CD8 T cells enter infected tissues and move within tissues to find and kill virally infected cells or tumor cells

• CD8 T cell motility is a key feature of CD8 T cell function
  • Affected by extracellular matrix
  • Fibroblastic reticular cells
  • Integrins
  • Molecules (chemokines)
# T cell data (Two-photon microscopy)

<table>
<thead>
<tr>
<th>Source</th>
<th>Number of T cells</th>
<th>Type of T cell</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>5348</td>
<td>Naïve</td>
<td>Fricke et al., Tasmin et al.</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>828</td>
<td>Activated</td>
<td>Thompson et al.</td>
</tr>
<tr>
<td>Lung (Flu)</td>
<td>99</td>
<td>Activated</td>
<td>Mrass et al.</td>
</tr>
<tr>
<td>Lung (Lipopolysaccharide LPS)</td>
<td>194</td>
<td>Activated</td>
<td>Mrass and Cannon et al.</td>
</tr>
</tbody>
</table>
Metrics to study T cell motility

- Speed
  - Cell-based speed
  - Displacement speed
- Turning angle and dependence of speed on turning angle
- Tendency to persist moving at the same speed
- Directionality (meandering ratio)
- Confined time
- Root mean squared slope – motion type
Objectives

• Identify differences and similarities in T cell motion within the different tissues.
• Confirm trends or identify new trends that can be observed in all tissues
Cell-based speed

Cell-based speed = \frac{d_1 + d_2 + d_3 + d_4}{Elapsed \ time}
Cell-based speed

Lymph (6.1), Villi (4.6), Lung (Flu) (5.2), Lung (LPS) (4.1)

Cells move fastest in the lymph node followed by lung (Flu), villi, and lung (LPS).
P-values for cell-based speed comparisons computed with Mann-Whitney U test

<table>
<thead>
<tr>
<th></th>
<th>Lymph</th>
<th>Villi</th>
<th>Lung (Flu)</th>
<th>Lung (LPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph</td>
<td>1.0</td>
<td>&lt;2x10^{-16}</td>
<td>1.0</td>
<td>&lt;2x10^{-16}</td>
</tr>
<tr>
<td>Villi</td>
<td>&lt;2x10^{-16}</td>
<td>1.0</td>
<td>5.5x10^{-10}</td>
<td>0.18</td>
</tr>
<tr>
<td>Lung (Flu)</td>
<td>1.0</td>
<td>5.5x10^{-10}</td>
<td>1.0</td>
<td>1.3x10^{-7}</td>
</tr>
<tr>
<td>Lung (LPS)</td>
<td>&lt;2x10^{-16}</td>
<td>0.18</td>
<td>1.3x10^{-7}</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Displacement-based speed

\[
\text{Displacement-based speed} = \frac{d_{\text{total}}}{\text{Elapsed time}}
\]
Displacement-based speed

Lymph (3.9), Villi (3.1), Lung (Flu) (1.9), Lung (LPS) (1.1)

Displacement speed is fastest in the lymph node followed by the villi, lung (Flu), and lung (LPS).
P-values for displacement-based speed comparisons computed with Mann-Whitney U test

<table>
<thead>
<tr>
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<th>Lung (LPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph</td>
<td>1.0</td>
<td>1.0x10^{-14}</td>
<td>9.1x10^{-14}</td>
<td>&lt;2x10^{-16}</td>
</tr>
<tr>
<td>Villi</td>
<td>1.0x10^{-14}</td>
<td>1.0</td>
<td>4.1x10^{-3}</td>
<td>&lt;2x10^{-16}</td>
</tr>
<tr>
<td>Lung (Flu)</td>
<td>9.1x10^{-14}</td>
<td>4.1x10^{-3}</td>
<td>1.0</td>
<td>6.0x10^{-5}</td>
</tr>
<tr>
<td>Lung (LPS)</td>
<td>&lt;2x10^{-16}</td>
<td>&lt;2x10^{-16}</td>
<td>6.0x10^{-5}</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Tendency to persist at a speed
Meandering ratio

Meandering ratio = $\frac{\text{d}_{\text{total}}}{d_1 + d_2 + d_3 + d_4}$
Cells meander more in the lung compared to the lymph and villi.
Turning angle ($\theta$)

T cells in the lung show a peak near $160^\circ$ which can be attributed to a back and forth motion.
Dependence of speed on turning angle ($\theta$)

All tissues except the LPS-inflamed lung show a dependence of speed on angle. LPS-inflamed lung seems to contradict universal coupling.
Confined time and ratio

We denote the amount of time a T cell lingers in one location as confined time. Given a time $t_i$ and location $(x_i,y_i,z_i)$, we count the time difference between $t_i$ and $t_j > t_i$ as confined time if the difference $t_j - t_i$ is greater than 100 seconds and the distance between $(x_i,y_i,z_i)$ and $(x_j,y_j,z_j)$ is less than 5 microns. Once the cell exits (say at time $t_k$) the 5 micron radius centered about $(x_i,y_i,z_i)$, the cell is tracked anew and the confined time is computed from $t_k$ and location $(x_k,y_k,z_k)$. We call the ratio of confined time to the total time the T cell is tracked the confined ratio.
Confined ratio

Lymph (0.0), Villi (0.074), Lung (Flu) (0.14), Lung (LPS) (0.23)

Cells linger at locations in the lung compared to the lymph and villi.
If the distance between a cube center and the T cell center is less than 5 microns, the cube volume is assumed to be patrolled. We also connect each two successive cell positions with a straight line and assume the cell patrols volume along the straight line.
Patrolled volume per time (cubic microns per second)

Lymph (10.1), Villi (6.4), Lung (Flu) (5.2), Lung (LPS) (3.4)
# P-values for patrolled volume comparisons

<table>
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<tr>
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<tr>
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</tr>
<tr>
<td>Villi</td>
<td>&lt;2x10^{-16}</td>
<td>1.0</td>
<td>1.0</td>
<td>&lt;2x10^{-16}</td>
</tr>
<tr>
<td>Lung (Flu)</td>
<td>&lt;2x10^{-16}</td>
<td>1.0</td>
<td>1.0</td>
<td>2x10^{-5}</td>
</tr>
<tr>
<td>Lung (LPS)</td>
<td>&lt;2x10^{-16}</td>
<td>&lt;2x10^{-16}</td>
<td>2x10^{-5}</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Slope values less than 1.0 are associated with Brownian motion and slope values greater than 1.0 are considered superdiffusive (Levy walks or ballistic motion).
Summary

• We observed a correlation between speed and turning angle. However, we also identified an exception to the rule in the LPS-inflamed lung.

• Our analysis shows that T cells tend to persist at the same speed in all tissue types (especially for slow and fast speeds).

• T cells tend to move slower, meander more, linger at locations longer and exhibit back and forth motion within the lung. Moreover the mode of activation in the lung seems to play a role in T cell motility since we observed differences in the LPS-infamed lung and the influenza-infected lung.

• The volume patrolled by a T cell is dependent not only on its speed but also the turning angles it uses, its tendency to meander, and the amount of time the cell spends confined to a location.
Virtual cells

• Simulations can be performed over extended periods in complex geometries
• Based on empirical distribution of speeds and angles
• Incorporates dependence of speed and turning angle
• Incorporates speed dependence on previous speed frame
Speed distribution (frame based)

Angle distribution

![Graph showing speed distribution with two curves: one for Lymph empirical and one for Lymph virtual.](image1)

![Graph showing angle distribution with two curves: one for Lymph empirical and one for Lymph virtual.](image2)
Speed vs Turning Angle

Tendency to persist at a speed

![Graph showing speed vs turning angle](image1)

![Graph showing tendency to persist at a speed](image2)
Meandering ratio

Confined ratio
Volume patrolled
(cubic microns per second)

Motion type