

T CELL MOTILITY

CCR7 fuels and LFA-1 grips

Naive T cells migrate rapidly through the lymph node. A high-resolution look at the chemokine receptor CCR7 and integrin LFA-1 reveals that T cells remain highly responsive to their microenvironment via instantaneous tuning of chemokine-regulated actin flow and integrin-regulated adhesion.

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The integrin LFA-1 and chemokine receptor CCR7 are well known to shape critical events in the immune response to injury and infection, ranging from lymph node (LN) homing and intranodal migration to T cell–antigen-presenting cell interactions^{1–3}. Naive T cells migrate at high speeds (15 $\mu\text{m}/\text{min}$) through the LN, guided by the fibroblastic reticular cell network that is decorated with chemokine and integrin ligands thought to optimize the encounter of T cells with antigen-bearing dendritic cells. To achieve rapid migration, T cells must optimize the competing adhesive and tractive forces derived from the forest of ligands. Naive T cells lacking either LFA-1 or CCR7 display reduced intranodal speed^{4,5}, but how these players transmit information from the extracellular microenvironment to effect intracellular motility machinery is poorly understood. In this issue of *Nature Immunology*, sophisticated measurement by Hons et al. of in vivo morphometry and in vitro cytoskeletal dynamics reveals that naive T cells cruise smoothly through the LN facilitated by independent chemokine signals that act as the ‘accelerator’ to the actin ‘motor’ and integrins that engage a highly dynamic ‘clutch’⁶.

While it has long been understood that leukocyte motility requires input from chemokine receptors, integrin-dependent adhesion and reorganization of the actin cytoskeleton, understanding of the molecular coupling of these processes for locomotion has remained rudimentary. The basic principle of migration is that cells need to apply an internally generated force, usually actomyosin based, to generate traction against their extracellular environment for forward movement. Traction is often provided by integrin-mediated adhesion to extracellular substrates but can be integrin independent in three-dimensional confinement⁷. The efficiency of migration can be ‘tuned’ on the basis of the degree of force generated and the extent of adhesion; too much or too little of either inhibits migration (Fig. 1). The molecular delineation

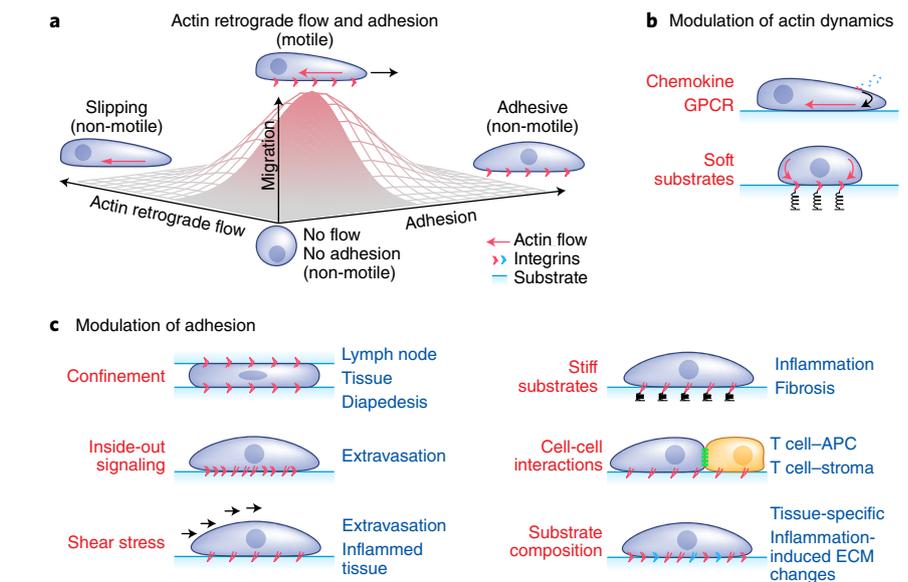


Fig. 1 | The balance of actin retrograde flow and adhesion regulates migration efficiency. **a**, Phase space diagram illustrating the cooperative behavior of actin retrograde flow and adhesion in promoting or inhibiting cell motility. The shaded region indicates the region investigated by Hons et al.⁶. **b**, Mechanisms that modulate actin retrograde flow include the addition of chemokines and cells encountering soft substrates. **c**, Mechanisms that modulate adhesion include confinement, inside-out signaling to activate integrins, cell–cell adhesion, compositional changes in the cell substrate, shear stress and stiffening of the local substrate. Blue font indicates in vivo examples of the physical processes associated with specific modes of modulating adhesion. GPCR, G protein-coupled receptor; ECM, extracellular matrix.

of these events has been greatly aided by new high-resolution imaging modalities that enable visualization of actin dynamics, membrane flow, force transmission and transmembrane receptor orientation during cell migration. Such approaches have been used to show that during T cell migration, intrinsic actin flow can ‘pull’ on integrin β -chain tails to activate LFA-1 and promote ligand binding⁸. This elegant mechanism would enable rapid ‘tuning’ of the speed of an actively migrating cell, enabling acute changes in actin dynamics to be quickly transmitted to enhance or reduce integrin-dependent traction.

Hons et al. build on those basic motility principles to revisit how CCR7 and LFA-1 shape the efficiency of T cell migration

through the LN⁶. It has been suggested that for cells to attain high-speed migration in the LN, chemokines drive T cell motility without the triggering of stable integrin-mediated adhesiveness⁴. Hons et al. shed new light on the way that T cells move within the LN in vivo by combining innovative high-resolution analysis of cell-shape changes (morphometry) with spatial–temporal migration parameters using intravital multiphoton microscopy⁶. Cell-shape change correlates with speed, such that the most elongated cells have the highest speed and rounded cells are slower. Remarkably, analysis of the temporal relationship between shape change and speed reveals that these events occur instantaneously. On the basis of such

morphodynamics, the authors propose that T cells migrate in a continuous sliding manner rather than a ‘caterpillar-like’ movement, in which shape and speed changes would be discontinuous due to cycles of protrusion and retraction. Sliding would be consistent with models in which T cell intranodal migration occurs independently of prolonged cycles of adhesion and de-adhesion. How then do CCR7 and LFA-1 affect this pattern of movement *in vivo*? Interestingly, CCR7 and LFA-1 appear to provide distinct contributions to cell shape and speed. Deletion of CCR7 affects shape, with considerable cell rounding, and reduces speed, while deletion of LFA-1 reduces speed but has little effect on shape. Notably, the effect of genetic deletion of both CCR7 and LFA-1 on cell shape and speed is additive, which suggests that chemokines and integrins control independent aspects of motility.

Mechanistically, Hons et al. find that *in vitro*, CCR7 signaling in the presence of the chemokine CCL19 controls a force-generating module by ‘tuning’ the speed of actin retrograde flow, independently of adhesion⁶. The CCR7-dependent elongated shape change during migration *in vivo* is directly linked to quantitative increases in actin flow; more CCL19 induces faster actin retrograde flow and more shape elongation. Chemokine signaling alone is unable to drive migration, leaving the ‘revved-up’ actin polymerization-generated forces to slip over the plasma membrane and the cell to run on the spot. In contrast, LFA-1–ICAM-1 engagement is sufficient to convert chemokine-driven retrograde actin flow into leading-edge protrusions and forward locomotion. LFA-1-mediated coupling of actin flow to the extracellular substrate occurs in the absence of discrete adhesive contacts with the substrate, which supports LFA-1’s ability to mediate force transmission through a dispersed network of integrins. Indeed, by ‘titrating down’ the availability of ICAM-1, Hons et al. elegantly demonstrate the exquisite sensitivity of such an integrin clutch, where partial clutch engagement and relatively poor force transmission can still support migration⁶. Thus, these novel findings demonstrate that in the absence of higher-order clustering, LFA-1 provides sufficient friction to enable T cells to continuously adapt to changing micro-anatomical environments without losing momentum.

Studies of this kind provide unique insight into the molecular roles of specific receptor–ligand interactions but have limitations due to the difficulty in translating the high-resolution *in vitro* data back into

in vivo studies to assess physiological effect in a complex environment. The resolution needed *in vivo* is simply not attainable with the current imaging modalities. Highlighting this challenge, at high resolution *in vitro*, distinct ‘walking’ and ‘sliding’ modes for T cell migration have been described, defined by the extent of the adhesion surface with the substrate⁹. Walking is associated with high speed and sequential, spatially distinct, small actin foci, while sliding correlates with slower speeds that result from a single contact zone on high-adhesive substrates. Such *in vitro* studies might at first glance appear inconsistent with the rapid, low-adhesive, continuous ‘sliding’ mode of migration suggested by Hons et al. through their *in vivo* morphometry⁶. While their morphometry analysis provides a first view into the dynamic shape changes T cells undergo when in motion, the dynamics of the integrins that mediate the interface between the T cell and its LN microenvironment remain unknown. T cells might lack large focal adhesion plaques, but the nimbleness afforded by highly dynamic micro-adhesive contacts might well underlie the *in vivo* continuous sliding behavior inferred from the cellular morphometric analysis. In this context, T cell intranodal migration might be more analogous to how a millipede smoothly locomotes over a changing terrain using many transient but necessary footholds. High-resolution quantitative imaging of integrin dynamics, in addition to the actin dynamics, will be needed to determine whether changes in the local microenvironment affect the mobility and activity of integrins to allow leukocytes to ‘tune’ the rate and efficiency of their migration.

Hons et al. contrast the independent roles of chemokines and integrins during high-speed, low-adhesive intranodal migration with the linear pathway invoked for the same molecules in leukocyte extravasation, in which chemokines are thought to be upstream regulators of integrin activation for adhesion in a process known as ‘inside-out signaling’⁶. From a biophysical viewpoint, these two perspectives most probably constitute different regions of a continuum that balances force generation and adhesion and allows leukocytes to ‘tune’ their response to diverse microenvironments⁷ (Fig. 1a). At the limits of each axis, migration is inhibited. With too little adhesion, cells cannot generate any protrusive forces; with too much adhesion, cells effectively get stuck in place. Similarly, too little actin retrograde flow means insufficient force generation; too much flow causes the integrin bonds to break before they can effectively engage the substrate. The current study effectively probes the

first quadrant of this phase space (Fig. 1a, shaded area), demonstrating that the effects of adhesion and actin retrograde flow can independently affect migration. Other migratory phenotypes, such as chemokine-mediated arrest during extravasation, could coexist with this view by inhabiting other regions of the continuum, such as by promoting additional adhesion through inside-out signaling. It will be interesting to see if future experiments can probe the rest of this phase diagram.

As highlighted with precision in this current study, a simple chemokine–integrin functional unit (CCL19 and LFA-1) can account for efficient T cell migration by cell-intrinsic coupling of chemokine-boosted actin dynamics to integrin–substrate traction. However, *in vivo*, the complexity of the system calls for a multi-dimensional model (Fig. 1a). Multiple signals can feed into the model and shift the balance between actin dynamics and traction (Fig. 1b,c). External pathways that modulate leukocyte adhesion include the following: confinement (which increases the adhesion or friction surface of the cell); inside-out signaling (which enhances integrin activation); cell–cell interactions (the T cell–antigen-presenting cell synapse); shear forces (which affect the lifetime of the integrin–ligand bond); and the local composition and material properties of the tissue environment (which affect the lifetime of the integrin–ligand bond). Layered on top of that are changes to those external factors imposed by inflammatory signals. Cell-intrinsic set-points might also alter the requirements for, or sensitivity to, external signals. These distinct motility needs will differ between tissues, between immune cell types and within specific leukocytes, depending on their activation and/or differentiation status, such as use of the integrin $\alpha_v\beta_3$ instead of LFA-1 by effector T cells in inflamed tissues¹⁰; the chemokine independence of the transendothelial migration of memory T cells into grafts¹¹; and the expression of distinct chemokine receptors on specific effector cell subsets¹².

Delineation of the context- and cell-type-specific requirements for the migration of immune cells will probably identify a plethora of non-canonical migration modes, including adhesion-free and chemokine-free motility. Moreover, despite environmental and cell-imposed constraints, it is becoming apparent just how plastic immune cells are when it comes to modulating migration to fit changing local environments¹³. Such flexibility is highly desirable for efficient pathogen clearance across diverse tissues but is a difficult moving target for therapeutic intervention. □

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Published online: 18 May 2018
<https://doi.org/10.1038/s41590-018-0118-y>

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Competing interests

The authors declare no competing interests.