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CCR7 fuels and LFA-1 grips

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Abstract

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

The integrin LFA-1 and the 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 (APC) interactions^{1, 2, 3}. Naïve T cells migrate at high speeds (15µm/min) through the LN guided by the fibroblastic reticular cell (FRC) network that is decorated with chemokine and integrin ligands thought to optimize T cell encounter with antigen-bearing dendritic cells. To achieve rapid T cell migration, T cells must optimize the competing adhesive and tractive forces derived from the forest of ligands. Naïve 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 measures of *in vivo* morphometry and *in vitro* cytoskeletal dynamics by Hons *et al.* reveal naïve 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 clutch⁶.

While we have long understood that leukocyte motility requires input from chemokine receptors, integrin-dependent adhesion and reorganization of the actin cytoskeleton, our understanding of the molecular coupling of these processes to drive locomotion remains rudimentary. The basic principle of migration is that cells need to apply 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 3D confinement⁷. The efficiency of migration can be tuned based on the degree of force generation and the extent of adhesion: too much or too little of either inhibits migration (Fig 1). Molecular dissection 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

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receptor orientation during cell migration. Such approaches were recently 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 cell speed in an actively migrating cell, enabling acute changes in actin dynamics to be quickly transmitted to enhance or reduce integrin-dependent traction.

Hons *et al.*⁶ build on these basic motility principles to revisit how CCR7 and LFA-1 shape the efficiency of T cell migration through the LN. To attain high-speed migration in the LN, it has previously been suggested that chemokines drive T cell motility without the triggering of stable integrin-mediated adhesiveness⁴. Hons *et al.*⁶ shed new light on the way in which 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 intra-vital multiphoton microscopy. Cell shape change correlated with speed, such that the most elongated cells had the highest speed and rounded cells were slower. Remarkably, analysis of the temporal relationship between shape change and speed revealed these events occurred instantaneously. Based on such morphodynamics, the authors⁶ propose that T cells migrate in a continuous sliding manner rather than a ‘caterpillar-like’ movement, in which shape and speed changes would have been discontinuous due to cycles of protrusion and retraction. Sliding would be consistent with models of T cell intranodal migration occurring independent of prolonged cycles of adhesion and de-adhesion. How then do CCR7 and LFA-1 impact this pattern of movement *in vivo*? Interestingly, CCR7 and LFA-1 appear to have distinct contributions to cell shape and speed. CCR7 deletion impacted shape, with significant cell rounding, and reduced speed, while LFA-1 deletion reduced speed but had little impact on shape. Importantly, the effect of genetic deletion of both CCR7 and LFA-1 on cell shape and speed was additive, suggesting chemokines and integrins control independent aspects of motility.

Mechanistically, *in vitro*, Hons *et al.*⁶ found that CCR7-signaling in the presence of the chemokine CCL19 controls a force-generating module by tuning the speed of actin retrograde flow, independent of adhesion. The CCR7-dependent elongated shape change during migration *in vivo* was directly linked to quantitative increases in actin flow; more CCL19 induced faster actin retrograde flow and more shape elongation. Chemokine signaling alone was 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 was 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 occurred in the absence of discrete adhesive contacts with the substrate, supporting 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.*⁶ elegantly demonstrated the exquisite sensitivity of such an integrin clutch, where partial clutch engagement and relatively poor force transmission can still support migration. 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 played by 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 test physiological impact in a complex environment. The resolution needed *in vivo* is just not attainable with current imaging modalities. To highlight 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 was associated with high speed and sequential spatially distinct small actin foci, while sliding correlated with slower speeds resulting from a single contact zone on high-adhesive substrates. These *in vitro* studies may at first glance appear inconsistent with the rapid low-adhesive continuous 'sliding' mode of migration implicated by Hons *et al.*⁶ *in vivo* morphometry. While their morphometry analysis provides our first view into the dynamic shape changes T cells undergo when in motion, the dynamics of the integrins mediating the interface between the T cell and its LN microenvironment remain unknown. T cells may lack large focal adhesion plaques, but the nimbleness afforded by highly dynamic micro-adhesive contacts may well underlie the *in vivo* continuous sliding behavior implied from the cellular morphometric analysis. In this regard, T cell intranodal migration may be more analogous to how a millipede smoothly locomotes over a changing terrain, utilizing 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 impact the mobility and activity of integrins to allow leukocytes to tune the rate and efficiency of their migration.

Hons *et al.*⁶ contrast the independent roles for chemokines and integrins during high speed, low adhesive intranodal migration to the linear pathway evoked for the same molecules in leukocyte extravasation, where chemokines are thought to be upstream regulators of integrin activation for adhesion in a process known as inside-out signaling. From a biophysical viewpoint these two perspectives most likely constitute different regions of a continuum that balances force generation and adhesion and allows leukocytes to tune their response to diverse microenvironments (Fig. 1a)⁷. At the limits of each axis, migration is inhibited. Too little adhesion and cells cannot generate any protrusive forces; too much adhesion and 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 impact migration. Other migratory phenotypes, such as chemokine-mediated arrest during extravasation, could coexist with this view by inhabiting other regions of the continuum, for example 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 modulating leukocyte adhesion include: confinement (increasing the

adhesion or friction surface of the cell), inside-out signaling (enhancing integrin activation), cell-cell interactions (T cell-APC synapse), shear forces (affecting the lifetime of the integrin-ligand bond), the local composition and material properties of the tissue environment (affecting the lifetime of the integrin-ligand bond). Layered on top, will be changes to these external factors imposed by inflammatory signals. Cell intrinsic set-points may 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 effector T cell use of $\alpha_V\beta_3$ instead of LFA-1 in inflamed tissues¹⁰; chemokine-independence for memory T cell transendothelial migration into grafts¹¹; and distinct chemokine receptor expression on specific effector subsets¹².

Dissection of the context and cell type-specific requirements for immune cell migration is likely to uncover 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 a difficult moving target for therapeutic intervention.

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Figure 1. The balance of actin retrograde flow and adhesion regulates migration efficiency.

(a) 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 addition of chemokine 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.