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Interstitial leukocyte migration and immune function

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The trafficking of leukocytes into and within lymphoid and peripheral tissues is central to immune cell development, immunosurveillance and effector function. Interstitial leukocyte trafficking is the result of amoeboid polarization and migration, guided by soluble or tissue-bound chemoattractant signals for positioning and local arrest. In contrast to other migration modes, amoeboid movement is particularly suited for scanning cellular networks and tissues. Here, we review mechanisms of leukocyte migration and sensing involved in diapedesis, tissue-based interstitial migration and egress, immune cell positioning in inflammation, and emerging therapeutic interference strategies.

An efficient immune reaction requires leukocytes to be at the right place at the right time. Nearly all steps, from maturation to activation and effector function, depend upon leukocyte migration and positioning in lymphoid and non-lymphoid tissues[1]. Both innate and adaptive immune functions depend upon interstitial leukocyte migration[2–5]. After leaving the bone marrow by way of the blood, monocytes and granulocytes reach lymphoid or peripheral tissues, move toward their targets and execute effector functions. T lymphocytes emigrate from the thymus, become activated by a cascade of cell-cell interactions in secondary lymphoid organs and circulate to peripheral tissues for effector function. Similarly, B lymphocytes move within secondary lymphatic tissues to capture antigen, receive T cell help and recirculate and become resident in the bone marrow and other lymphoid organs as antibody-secreting plasma cells. Leukocyte entry into tissue through the vascular endothelium has been studied in detail (reviewed in refs. 1,6), whereas the mechanisms of leukocyte diapedesis into and navigation within and out of tissues are less clear. The density and position of leukocytes in tissues is a steady-state result of influx and migration, local activation, proliferation and death, and efflux (**Supplementary Fig. 1** online), which together confers effector functions. Efficient pathogen elimination and resolution can be reached by either subthreshold non-inflamed immune surveillance or nondestructive short-term inflammation[7]. Persisting antigen, however, may lead to chronic inflammation, tissue remodeling and formation of *de novo* structures, such as tertiary lymphatic tissues or granulomas. In chronic inflammation, stepwise tissue destruction is followed by defective healing. Because of their role in mediating inflammatory reactions, interstitial migration and positioning of leukocytes represent therapeutic targets for either suppressing unwanted inflammation or boosting insufficient host responses[8].

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Mechanisms of amoeboid migration in leukocytes

Leukocytes use amoeboid cell migration mechanisms. Reminiscent of the amoeba *Dictyostelium discoideum*, polarized leukocytes develop a small leading edge consisting of short-lived pseudopods, followed by the cell body that contains the nucleus, and a posterior, near-cylindrical tail of 2 to 10 μm in length termed the uropod (**Fig. 1a**,**b**). Four steps mediate the amoeboid migration cycle: the leading edge protrudes one or several pseudopods by actin flow, protruding membrane and surface receptors interact with the substrate, actomyosin-mediated contraction of the cell body occurs in mid-region, and so the rear of the cell moves forward. These steps occur in a cyclic manner, generating forward movement.

Amoeboid migration is very different from mesenchymal or collective migration modes employed by other cell types in three-dimensional models[9]. Amoeboid migration is used by leukocytes (and likely many stem cells), is fast (up to 30 μm/min), lacks strong adhesive interactions to the tissue and commonly preserves tissue integrity rather than degrading it[10]. Mesenchymal migration used by fibroblasts, smooth muscle cells and cancer cells is much slower (less than 1 μm/min), generates stronger adhesion sites and causes proteolytic extracellular matrix (ECM) remodeling[11]. Collective cell migration is used by cells that retain their cell-cell junctions, remodel ECM, and move either as a two dimensional sheet, three-dimensional strand or compact cluster through tissue[12], as during branching morphogenesis, vascular sprouting and epithelial wound closure[13]. Compared to other migration types, amoeboid movement is special because it supports a uniquely fast capability to sense and integrate signaling input from the extracellular environment ('scanning')[14].

After polarization, the leading edge contains rapidly forming and rebuilding networks of filamentous actin that include abundant membrane ruffles indicative of dynamic probing of the environment. The leading edge is particularly sensitive to receptor engagement, including that by Fc receptors (FcRs), T cell antigen receptors (TCRs), chemokine receptors[15] and β_2 integrins in intermediate or active state[16], all of which

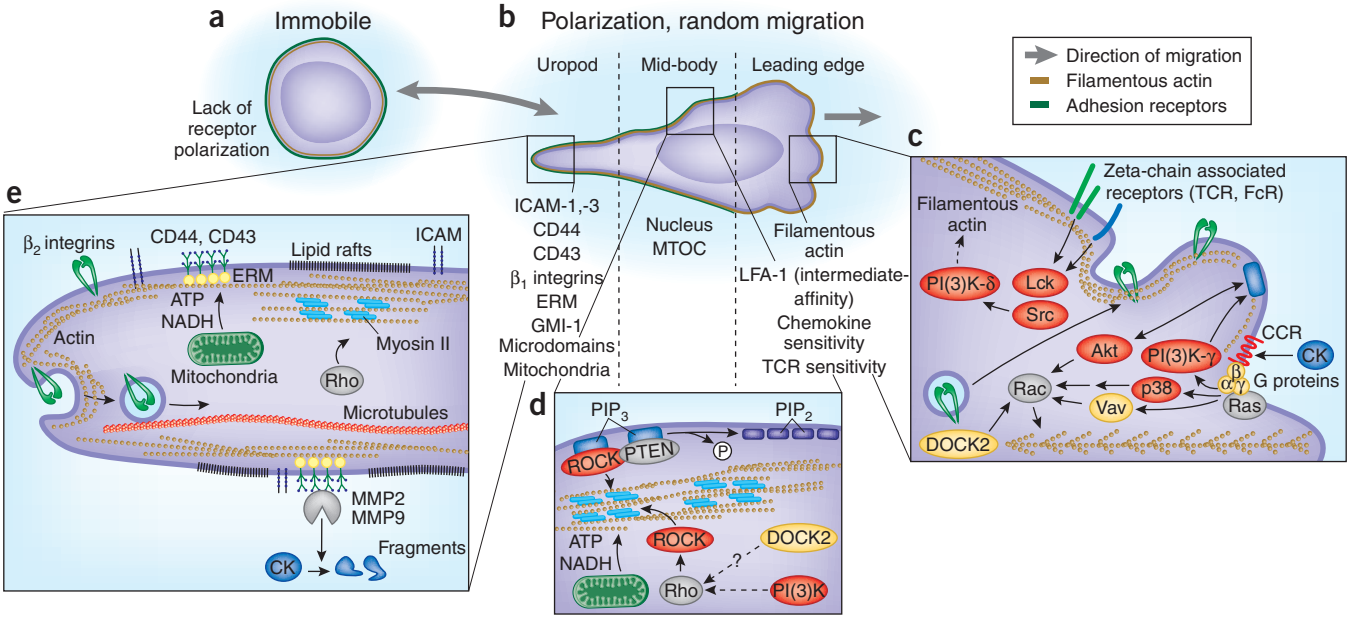

Figure 1 Morphology, surface receptors and signaling in amoeboid leukocyte migration. (**a**) Round morphology of immobile or freely floating leukocyte, including uniform distribution of surface receptors and cytoskeleton. (**b**) Amoeboid shape after polarization during random migration and chemotaxis. (**c**) Surface receptors, cytoskeletal structure and signaling events in the leading edge, lateral portion of the cell body and uropod. Class I PI(3)Ks are lipid kinases that phosphorylate phosphatidylinositol-(3,4)-bisphosphate (PIP_2) to phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3). PIP_3 is thought to form multimers at the inner leaflet of the plasma membrane that serve as docking and activation site for pleckstrin-homology (PH) domain–containing proteins, including Akt (protein kinase B). Other pathways directly and indirectly triggered by chemoattractant receptors are Ras and p38 and DOCK2. As central effector of these pathways, Rac mediates the formation of actin filaments that move the plasma membrane forward. Other surface receptors, including TCR and FcR, activate the tyrosine kinases Lck and Src and downstream PI(3)K-δ. (**d**) The mid-portion contains actomyosin filaments that are under the control of Rho and ROCK. (**e**) The uropod forms an adhesive and contractile rear that contains microtubules and mitochondria. CK, cytokine; ERM, adaptor proteins of the ezrin-radixin-moesin family; GM-1, monosialotetrahexosylganglioside; MTOC, microtubule-organizing center; Vav, Vav-family guanine-nucleotide exchange factor.

serve to initiate contact with other cells, induce signal transduction[17] and mediate phagocytosis after binding of bacteria and other particles[18]. If normalized to membrane area, the density of most surface and chemoattractant receptors on the leading edge is equal to that on other cell parts, at least at the light-microscopic level[19,20], but at ultrastructural resolution, other receptors, such as β_2 integrins in neutrophils, show discrete relocation toward the tips of ruffles[21]. The mid-region of amoeboid cells contains the nucleus and a relatively immobile cell region that maintains the front–rear axis. The trailing edge contains the highly glycosylated surface receptors CD43 and CD44, adhesion receptors including intercellular adhesion molecule (ICAM)-1, ICAM-3, β_1 integrins and ERM adaptor proteins, as well as GM-1-type cholesterol-rich microdomains[22]. The uropod mediates cell–matrix and cell–cell interactions during migration and has a putative anchoring function[10].

Polarization of cytoskeletal and signaling scaffolds

At the leading edge (**Fig. 1c**), pseudopod and lamellipod protrusion occurs either spontaneously or induced by migration-promoting factors binding to surface receptors. In leukocytes, polarization and migration to chemoattractant gradients, known as chemotaxis, are induced by various compound classes, including chemokines and cytokines, lipid mediators, bacterial factors and ECM degradation products including fragments of collagen, fibronectin and elastin[23–25]. Many chemoattractants transmit signals through heterotrimeric G-protein-coupled receptors (GPCRs). In leukocytes, most GPCRs transmit through the α subunit of $G_{i\alpha}$, the subtype of G protein that can be inhibited by pertussis toxin. These GPCRs include the fMLP (*N*-formyl-Met-Leu-Phe) receptor and C5a receptor; chemokine receptors including CCR7, CXCR4, CXCR5 and CCR3; the leukotriene B4 receptor BLT1; sphingosine-1-phosphate receptors 1–4 ($S1P_{1-4}$) and lysophosphatidic acid (LPA) receptors 1–3 (ref. 26). All these GPCRs mediate promigratory signals but also enhance cell activation. A key GPCR-mediated pathway is signaling through class Ib phosphatidylinositol-3-kinase (PI(3)K), containing the p110γ catalytic subunit). PI(3)K-γ is recruited to the inner leaflet of the plasma membrane by the G protein βγ subunit, where it becomes activated, and then phosphorylates phosphatidylinositol phosphates (PIPs) and other effectors (reviewed in ref. 27). PIPs serve as docking sites for pleckstrin-homology domain–containing proteins, notably Akt (also known as protein kinase B), which is implicated in inducing actin polymerization and pseudopod protrusion by phosphorylating downstream effectors[28], such as the actin-binding protein girdin[29]. A second pathway linked to PI(3)K activation is induced by ζ-chain-associated receptors, including TCRs and FcRs. These receptors signal through tyrosine kinases Lck and Zap-70 to class Ia PI(3)Ks (consisting of p110δ) and activate downstream Akt, as well as the GTPases Rac and Cdc42 (ref. 30). A third, PI(3)K-independent pathway induced by the fMLP receptor in neutrophils leads to the activation of p38 mitogen-associated protein kinase and downstream Rac activation[7]. Lastly, the Rac guanine nucleotide exchange factor DOCK-2 leads to Rac activation at the leading edge[31]. Ultimately, Rac induces actin polymerization through WAVE (Scar) and Arp2/3. WAVE, a member of the WASP family of actin-binding proteins, mediates actin filament formation[32]; Arp2/3 causes sideward branching of actin filaments. Together, these activities generate interconnected, branched networks[33]. Thus, promigratory signals received at the leading edge generate local Rac activation and actin network protrusion, pushing the plasma membrane outward. The mechanisms of preferential receptor sensitivity at the leading edge are likely diverse and may include local signal amplification mechanisms[34] and the exclusion of counter-regulatory proteins.

The mid-region (**Fig. 1d**) generates actomyosin-based stiffness and contractility, limits lateral protrusions and thereby maintains a stable, bipolar cortex. The cytoskeletal motor protein myosin II located in the central and rear regions of leukocytes promotes actin filament contraction and limits lateral protrusions. Myosin II cross-links actin filaments in parallel, forming the contractile shell required to hold the extending cell together and propelling the cell nucleus, the most rigid part of the cell, forward[35]. Inhibition of myosin II in neutrophils leads to ectopic lamellae on two-dimensional substrates[36] or the loss of rear-end retraction in three-dimensional tissues[37], resulting in impaired migration. Upstream of myosin II, by yet unclear mechanisms, PI(3)K-γ and possibly DOCK-2 suppress lateral protrusions[38]; deletion of either protein enhances cell turning such that overall cell mobility is compromised[31]. The phosphatase PTEN also contributes to lateral stability by preventing ectopic protrusion formation[7]. PTEN is excluded from the leading edge but active in lateral and rear cell parts, where it dephosphorylates kinases, including PI(3)K and Akt, as well as phosphatidylinositol-(3,4,5)-trisphosphate, and thereby counteracts protrusion formation[7,39].

The uropod (**Fig. 1e**) extends rearward from the nucleus and contains the microtubule-organizing center and rearward-polarized microtubules, the Golgi, and abundant actin-binding ERM proteins. In association with microtubules, mitochondria localize to the rear of the cell, which, presumably owing to local ATP delivery to the region of ATP-dependent actomyosin contraction, is required for proper polarization, uropod retraction and migration[40]. Amoeboid polarization thus generates a bipolar mechanosensory state with a dynamic leading edge to acquire new contacts and signals, a stiff mid-body, and a sticky uropod that is dragged along the substrate and stabilizes the cell position in complex environments[10,41].

Leukocyte migration in different environments

Leukocytes are able to migrate along or through most, if not all, tissues of the body. Both two-dimensional surfaces, such as inner vessel walls, the peritoneum and the pleura, and three-dimensional tissues, composed of mostly cellular (lymph node) or fibrillar ECM components, serve as 'substrates' for this migration (**Fig. 2**). As part of the amoeboid migration program, leukocytes use adhesion receptor–dependent mechanisms, known as haptokinesis, for migration across two-dimensional surfaces. Migration across two-dimensional ECM or an endothelial surface requires integrin-mediated attachment and polarized adhesion, notably through binding of integrins $\alpha_4\beta_1$ and LFA-1 to counterpart ligands VCAM-1 and ICAM-1 on the endothelial cell (**Fig. 2a**)[6]. By contrast, in three-dimensional ECM environments, cells use weakly adhesive to nonadhesive interaction and traction mechanisms. Leukocyte migration within interstitial tissue *in vitro* and *in vivo* is integrin independent, being mediated instead by actin flow along the confining ECM scaffold structure, shape change, and squeezing (**Fig. 2b**)[10,22,37]. In contrast to two-dimensional migration, the three-dimensional tissue network confines and mechanically anchors cells from all sides so they intercalate alongside and perpendicular to tissue structures[10,37,42]; this differs from integrin-dependent mesenchymal and other migration modes[9]. How leukocytes distinguish substrate anatomy—that is, two-dimensional versus three-dimensional environments—and adjust their adhesion requirements is presently unknown.

Pericellular proteolysis

The degradation of ECM is a key mechanism supporting cell migration through physically constraining tissue regions[43]. The penetration of the very dense meshwork of basement membranes likely requires proteolytic activity, at least during the initial phase of leukocyte influx[6,44,45]. Whether interstitial leukocyte migration *in vivo* depends upon similar proteolytic ECM degradation is unclear, as controversial results have been reported[10]. Better dissection of the *in vivo* requirements for proteolytic ECM processing in different phases of leukocyte trafficking will require knowledge of ECM structure and composition before and after trafficking and consideration of other protease functions that may affect migration rates through diverse mechanisms, including the processing of surface receptors, cytokines, and growth factors[46,47]. In defined *in vitro* environments, such as fibrillar three-dimensional collagen matrices of known pore diameter (averaging 3 to 6 μm), lymphocytes and myeloid cells migrate in the absence of collagen degradation or proteolytic track generation[10]. As a mechanism, nonproteolytic amoeboid migration results from marked shape change, propulsion and squeezing through narrow tissue regions (**Fig. 2b**), similar to leukocyte movement through interstitial tissue with minimum collateral damage during nondestructive inflammation[10,41,48]. Because the physical structure of interstitial human and mouse tissues *in vivo* is quite heterogeneous, ranging from loose fibrillar regions of spacing similar to that of three-dimensional collagen lattices to compact dense connective tissue with submicron spacing (K. Wolf and P.F., unpublished data), it is likely that leukocytes adapt to tissue geometry and follow paths of least resistance, a process known as contact guidance.

Migration programs in different leukocytes subsets

The basic amoeboid migration program is retained in most, if not all, leukocytes as well as lymphoma cells[49]. Whereas granulocytes and lymphocytes maintain the typical 'hand-mirror' shape reminiscent of *Dictyostelium*, monocytes contain more cytoplasm and often lack a bona

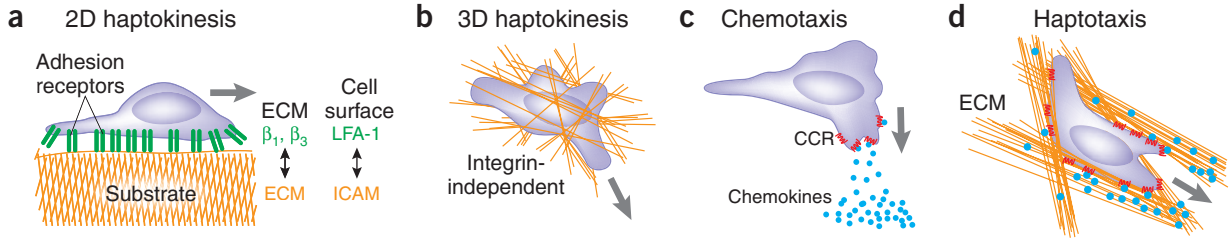

Figure 2 Principles of leukocyte–substrate interactions and guidance. (**a**) Haptokinetic, adhesion-dependent migration across a two-dimensional (2D) surface. Two-dimensional leukocyte migration requires integrin-mediated interaction with the substrate. *In vivo*, haptokinetic migration is present during leukocyte crawling across vascular endothelium or inner body surfaces, such as the peritoneum or the bronchial tract. (**b**) Three-dimensional (3D) haptokinesis and contact guidance results from shape change and the flow of anterior actin, but in leukocytes, lacks integrin-mediated adhesion requirements. In organized tissue, alignment of the cell body to nonrandom tissue structures leads to contact guidance. (**c**) Chemotaxis is induced by soluble, freely diffusing compounds that lead to preferential signaling and actin-rich protrusions at the leading edge. Chemotaxis adds a direction to 2D and 3D haptokinetic migration. (**d**) Haptotaxis is the directed migration toward chemoattractants that are immobilized on tissue structures, such as interstitial collagens or a stromal cell network.

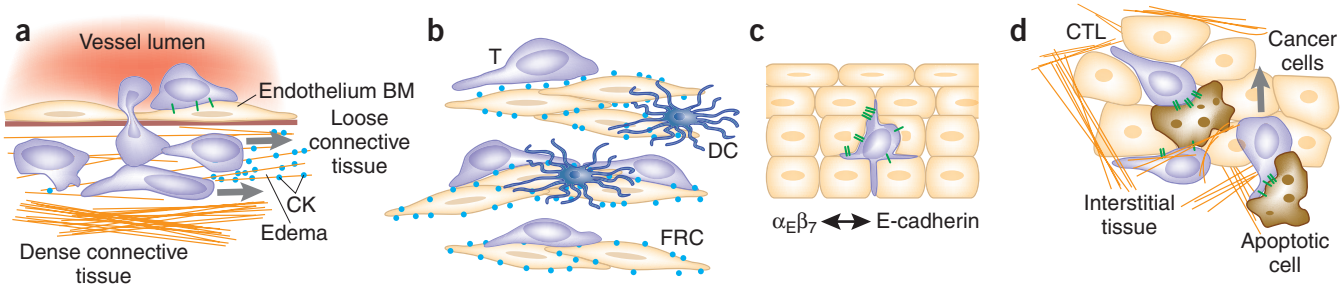

Figure 3 Leukocyte migration in different environments *in vivo*. (**a**) Interstitial leukocyte migration in loose connective tissue. Such preformed tracks of least resistance are present along most basement membranes (BMs), including those lining blood vessels and below epithelia that are particularly receptive to edematous swelling and enlargement of ECM gaps and tracks[41]. (**b**) Migration along cellular networks in lymphoid organs, such as fibroblastic reticular cells[63] (FRCs) or DC networks[64] in the T cell zone in lymph nodes. (**c**) Intraepithelial migration and positioning. After reverse transmigration through the basement membrane, leukocytes migrate briefly along cell–cell junctions until stable positioning is reached. In epidermis, integrin $\alpha_E\beta_7$ in lymphocytes is the counterpart receptor for epithelial E-cadherin. (**d**) Intratumoral migration guided by cell–matrix and cell–cell contacts. CK, cytokine.

fide uropod, but otherwise follow amoeboid principles of shape change and squeezing[10]. In dendritic cells (DCs), the leading edge consists of multiple dendrites that intercalate between tissue structures, but the cell body and uropod are retained[37,50]. As perhaps the only exception, tissue-resident macrophages and macrophage-derived epithelioid cells adopt adhesive fibroblast-like morphology[51], are poorly motile and show upregulated β_1, β_2 and β_3 integrins, together with matrix metalloproteases (MMPs)[52,53]. Whereas the principles of amoeboid movement, including basic adhesion, protease and signaling requirements, seem to apply to all rapidly moving leukocytes, their peak velocities *in vitro* and *in vivo* differ for neutrophils (up to 30 μm/min), lymphocytes (up to 25 μm/min), B cells (15 μm/min), DCs (10 μm/min) and monocytes (5 μm/min). The maximum migration velocity is likely proportional to myosin II activity and the deformability of the nucleus, as suggested by impaired migration rates *in vitro* because of nuclear entrapment at ECM structures after myosin II inhibition[37]. Further, the repertoire of expressed chemoattractant and adhesion receptors mediating migration and attachment diverge, providing cell type–specific recruitment and positioning in tissues[1,8,26,31].

Leukocyte trafficking into tissues and initial migration

For immigration of circulating leukocytes into tissues (**Fig. 3**), transmigration through the vascular endothelial layer is the rate-limiting step, involving two independently regulated events, binding to vessel endothelium followed by diapedesis[1,54]. The basement membrane underlying all normal vessels represents the first postendothelial tissue structure and barrier to cells undergoing diapedesis (**Fig. 3a**). The known mechanistic steps of vascular basement membrane penetration include adhesion to the substrate via integrin $\alpha_6\beta_1$, the principal laminin receptor on leukocytes[55]. For passage through vessel basement membrane in interstitial tissue, locally confined cleavage of the structural proteins laminin-10 and type IV collagen occurs by secreted or membrane-anchored MMPs and serine proteases[44,56]. Cell body deformation is coupled to cytoplasmic propulsion and streaming through preexisting or newly formed pores.

In acute inflammation *in vivo*, early neutrophil diapedesis occurs preferentially through basement membrane regions having decreased density of laminin-10, collagen IV and nidogen-2, suggestive of anatomic zones of least resistance between the pericytes[45]. As leukocyte influx and proteolytic ECM degradation proceed, these regions further lose basement membrane components and develop into inflammation-induced preferred entry points for other leukocytes[6,45]. In brain vessels, proteolytic basement membrane remodeling is essential for immune cell diapedesis[57,58], but it remains unresolved whether proteolytic breakdown is indispensable or rather auxiliary for diapedesis through other basement membranes[44,45,59]. The deformation capability of leukocytes is considerable, reaching submicron constrictions in neutrophils[60]; thus, proteolytic and nonproteolytic mechanisms may synergize.

In transendothelial migration models *in vitro*, the engagement of CD31 and likely of integrin β_2 in neutrophils and activated lymphocytes leads to the upregulation of integrin $\alpha_6\beta_1$ and $\alpha_4\beta_1$, thus initiating a tissue phenotype that supports interstitial and cell-guided crawling[55,61,62]. Upon leukocyte diapedesis, while the uropod is still engaged with the vessel wall, the leading edge reaches the perivascular loose connective tissue, consisting of filamentous collagen fibers and elongated gap-like tracks that facilitate cell alignment and migration (**Fig. 3a**). Such perivascular tracks are present in virtually all organs and provide preferential cell migration routes to distal regions of the tissue[41]. With inflammation, these tracks become further widened by hydrostatic pressure from vascular leakage and edema, likely facilitating cell trafficking[41]. In inflamed tissue, freely diffusing chemoattractants, such as bacterial peptides, provide short-lived or pulsatile directional information (**Fig. 2c**), in addition to the longer-lived cues provided by constitutive or induced tissue-bound chemoattractants (**Fig. 2d**)[26]. Thus, besides chemotaxis, haptotaxis and contact guidance synergize to direct early steps of interstitial trafficking.

Migration along cell scaffolds

In contrast to epithelial and mesenchymal cells, leukocytes are particularly prone to use other cell surfaces for guided migration, recognition and arrest as part of their immune surveillance function (**Table 1**). In uninflamed lymph nodes, T and B lymphocytes immigrate through high endothelial venules and predominantly migrate along the cellular network of fibroblastic reticular cells (FRCs), DCs and follicular dendritic cells, as shown by intravital microscopy in living lymph nodes (**Fig. 3b**)[63,64]. FRCs in particular provide a structural scaffold, with cell-surface ICAM-1 as a counter-ligand to leukocyte LFA-1 and also with decoration with chemokines[63]. T cell positioning is dependent on CCR7 and its ligands CCL21 and CCL19, expressed by and bound at the surfaces of FRCs and DCs[63]. Mice lacking CCR7 or its chemokine ligands fail to generate proper T and B cell regions[65,66]; thus, both lymphocyte positioning and the anatomy of the T and B cell zones largely depend upon chemokine guidance. The migration pattern up and down the FRC network corresponds to a persistent random walk[2–4], suggesting haptokinesis and/or contact guidance[2,63,67]. Besides promoting migration, chemokine signals submit latent preactivation that increases

Table 1 Haptokinetic and adhesive cell-cell interactions in interstitial leukocyte trafficking

Cellular scaffold	Ligand contacted	Leukocyte	Receptor	Outcome	Ref.
Fibroblast, synoviocyte	LFA-1	All	ICAM-1	Slowing, arrest	6
	FN	All	$\alpha_5\beta_1$, $\alpha_4\beta_1$	Slowing, arrest	138
	VCAM-1	All	$\alpha_4\beta_1$	Slowing, arrest	6
	CD40	T	CD40L	Costimulation	139
DC	ICAM-1	$CD4^+$, $CD8^+$	LFA-1	Slowing, arrest	70
	CCL21	$CD4^+$, $CD8^+$	CCR7	Costimulation, arrest	63
MZ stromal cell	ICAM-1	MZ B	LFA-1	Arrest	76
	VCAM-1	MZ B	$\alpha_4\beta_1$	Arrest	76
FDC	CXCL13	B	CXCR5	Haptotaxis	66
LEC (lymph node)	S1P	T, B	$S1P_1$	Emigration from lymph node	91
Afferent LEC	ICAM-1	T, DC	LFA-1	Arrest, impaired tissue egress	95
	VCAM-1	T, DC	$\alpha_4\beta_1$	Arrest, impaired tissue egress	95
	CLEVER-1	T, DCs	?	Tissue egress	98
	Macrophage mannose receptor-1	T, DCs	?	Tissue egress	97
FRC	ICAM-1	T, B	LFA-1	Arrest	140
	CCL19, CCL21	T, B, DC	CCD7	Haptotaxis, costimulation	63
	ER-TR7 epitope	T, B, DC	?	Haptotaxis?	141
Target cell	Peptide–MHC I, ICAM	CTL	TCR, LFA-1	Arrest, effector function	108–110
	E-cadherin	CTL	$\alpha_E\beta_7$	Arrest, polarization, killing	120
Epidermis	E-cadherin	γδ T	$\alpha_E\beta_7$	Adhesion, long-lived integration	81

MZ, splenic marginal zone.

T cell sensitivity to subsequent activation, such as TCR triggering[68]. Despite their importance for cell-cell interactions and migration across surfaces[16], no active role for LFA-1–ICAM-1 interaction in the intranodal migration of T cells, B cells and DC has been detected[37,69]. Other mechanisms providing translocation along cell scaffolds may include weak adhesion by chemokine–chemokine receptor interaction or nonadhesive shape change[37,69]. Given cell scaffold–mediated migration strategies in lymph nodes, it is likely that leukocytes in peripheral tissues move along stromal cells as part of their surveillance function[61].

Balancing adhesion and migration

Trafficking leukocytes often reduce their migration speed, pause and polarize toward the bound cell or tissue structure to execute crucial functions, including phagocytosis, cell-to-cell signaling and activation, and the release of cytokines or toxic factors toward a cell encountered. *In vivo* studies using histological end points cannot differentiate interstitial leukocyte accumulation by chemotaxis from random migration coupled to local arrest of migration and so provide only indirect insight into positioning mechanisms. Because of the current lack of data from live-cell imaging in peripheral tissues, little is known about the steady-state proportion of moving and sessile infiltrating cells during acute and chronic inflammation. Therefore, most knowledge of positioning stems from dynamic imaging in three-dimensional *in vitro* models and in lymph nodes *in vivo*.

At least three basic kinetic states govern leukocyte positioning in tissues, including fast migration (5 to 25 μm/min), slow and often locally confined movement (2 to 5 μm), and adhesive arrest; and these rapidly interconvert (**Fig. 4**). Based on these kinetic states, leukocyte accumulation in tissues occurs through at least three distinct mechanisms. Local engagement of adhesion receptors causes individual leukocytes to stick and become immobilized at a specific spot. The degradation of promigratory 'go' signals causes cell populations to slow down or stop movement. And the loss of exit signals confines cells to a local microenvironment despite ongoing migration.

Complete migration arrest is mediated by activation of adhesion receptors on the moving cell followed by attachment to counter-receptors on other cells or ECM structures, leading to an immobilized cell (**Table 1**)[70]. Within seconds, adhesion overrides ongoing promigratory signals; this is followed by cytoskeletal polarization toward the bound cell or ECM structure[70,71]. Despite the promigratory lymph node environment, antigenic signaling prompts T and B cells to cease migration through LFA-1–ICAM-1 interaction[70] followed by the formation of a stable immunological synapse[72]. Under conditions of strong antigenic signaling, T cells contacting DCs undergo a rapid and strong adherence response to the antigen-presenting DC for signal exchange[73–75]. Activated B cells after antigen uptake attach to follicular stromal cells through LFA-1–ICAM and integrin $\alpha_4\beta_1$–VCAM interactions and receive survival signals[76]. Besides cell–cell signaling, such stable binding further supports the polarized release of cytokines into the cell–cell junction, as in helper T cells bound to B cells[77]. By contrast, local confinement despite ongoing motility occurs by local adhesion coupled to slow migration 'on the spot'[72]. Here, integrin-mediated arrest is coupled to polarized cytoskeletal dynamics and movement across the surface of the counterpart cell; this movement is thought to support receptor turnover and scanning of the cell surface[3–5,14,74,78]. In both cases, the immobilization response is proportional to, and probably caused by, transient elevation of intracellular calcium in the T cell that confines migration by enhanced adhesion (**Fig. 4**)[5,74].

The adhesive contact structures mediating arrest vary. In T cells, the immunological synapse is mediated by an adhesion plane enriched in filamentous actin, LFA-1, TCR and costimulatory molecules[72]. In monocytes and immature dendritic cells, adhesive arrest to ECM substrate is mediated by podosomes composed of multiple actin-rich focal adhesion zones of 1 to 3 μm in diameter and 10 to 30 min duration, formed underneath cells adhering to two-dimensional ECM substrate[79]. Compared to migrating cells, both immunological synapses and podosomes are relatively stable and thus slow migration down or confer adhesive arrest for a limited duration, ranging from

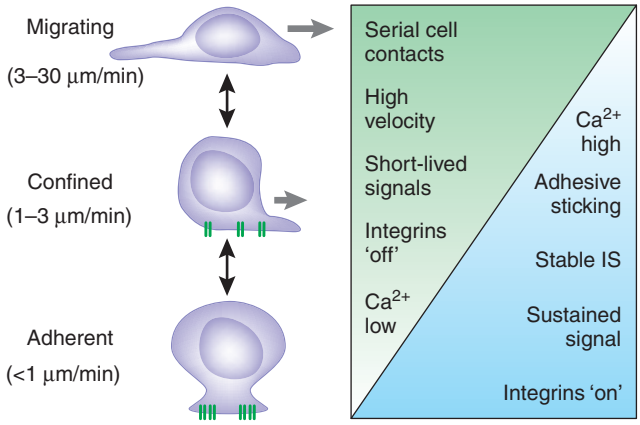

Figure 4 A balance between amoeboid migration, adhesive arrest and intermediate states. Moving leukocytes are able to slow down and completely cease migration within seconds to minutes and vice versa. High-velocity movement occurs by actin flow and shape change but without integrin-mediated adhesion and high calcium currents. Conversely, adhesive arrest is mediated by integrin engagement, accompanied by calcium oscillations and loss of amoeboid polarization. Because promigratory and antimigratory signals are gradual, rather than discrete, intermediate states may be adopted, resulting in slow migration in a confined region, often on the surface of a single cell. IS, immunological synapse.

minutes to hours. Thus, whereas arrest mechanisms and underlying signals and adhesion structures vary in a cell- and context-dependent manner, migration confinement is a graded response proportional to proadhesive signal strength.

Long-lived confinement and tissue residency

Under homeostatic conditions, long-lasting tissue residency (for days and weeks) of leukocytes supports sentinel and surveillance function at epithelial interfaces to the outside world or at inner surfaces that filter blood or lymph for pathogens[80]. Whereas the tissue lifespan of neutrophils until death is estimated at a few hours and that of interstitial T cells until egress is estimated at 1 d, resident macrophages, DCs or γδ T cells may survive months to years until egress or death. In the skin, intraepidermal γδ T cells integrate between keratinocytes and use integrin $\alpha_E\beta_7$ for binding to epidermal E-cadherin (**Fig. 3c**)[81]. Likewise, intraepithelial leukocytes in the gut are dependent on $\alpha_E\beta_7$ binding to E-cadherin[82]. For other tissue-resident leukocytes, such as interstitial DCs or macrophages, the retention mechanisms are unknown.

Relocation

After immigration and migration arrest, the re-onset of migration prompts leukocyte relocation toward regions nearby[54]. The lymph node is the best-studied organ for relocation; here, T and B cells change subregions in the course of their activation and differentiation program. After antigen capture, B cells located within the B cell follicle become activated, upregulate CCR7 and migrate chemotactically along a CCL21 gradient toward the follicle boundary, where they contact activated $CD4^+$ helper T cells[83]. Conversely, initially activated $CD8^+$ T cells expressing CCR5 are directed by CCL3 and CCL4 to reach the region of ongoing interaction between $CD4^+$ T cells and DCs, where they receive T cell help[84]. In lymphoid organs relocation connects particular leukocyte functions to sequentially occur in different anatomic regions; however, repositioning programs in peripheral tissues remain elusive.

Relocation further serves to allow lymphocytes to accumulate short-lived activation signals. Naive T cells migrate at high velocity across the surface of DCs presenting noncognate or very weak antigen and receive weak, homeostatic signals[2,74] (J. Storim and P.F., unpublished data). If antigenic strength is moderate, naive T cells slow down after engaging with the DCs, detach again within minutes and engage with neighboring DCs, where they receive TCR triggering signals[75]. Here, serial interactions with different DCs are mediated by relocation and provide pulsatile cognate TCR-mediated signals until early activation is reached, mediating T cell arrest and commitment to a single DC[4,75,85].

A special type of relocation during stable cell–cell adhesion is the 'carried translocation' of T cells bound to a moving DC[86], a resting B cell pushed by a moving T cell[71] and the guidance of T cells by an activated migrating B cell[83]. Because of their stability, these conjugates provide antigenic signaling and passive translocation of immobile cells to another tissue region, yet the function of these interactions remains unclear. Thus, both active and passive cell movements contribute to leukocyte relocation.

Counter-regulation of interstitial leukocyte trafficking

Beyond adhesive migration arrest, the local reduction of promigratory signals is achieved by the down-modulation of chemoattractant receptors, receptor desensitization and ligand competition, whereas termination of chemoattractant activity occurs through capture by neutralizing chemoattractant receptors and/or proteolytic degradation. After ligation, chemoattractant receptors become internalized and either recycled to the leading edge or stored in vesicles in the uropod, thus limiting the availability of both the chemoattractant and its receptor[20]. In T cells, the activation-induced downregulation of $S1P_1$ is the best-studied example of how chemoattractant signaling is tuned (see below)[87]. Migration-inducing agonists can further be competed away from the receptor by endogenous antagonists. Resolvin E1 acts as a competitive antagonist of LTB4 by binding to and desensitizing BLT1 (ref. 88). Resolvins are found in high concentrations in inflammatory tissue fluids, where they counteract the influx of neutrophils by limiting LTB4-mediated chemotaxis and enhance the resolution of dermal inflammation, peritonitis and colitis in mouse disease models[89]. Local chemokine availability is reduced by binding to chemokine receptors on bystander cells ('sink function'). Erythrocytes and endothelial cells express silent chemokine receptors, including DARC (Duffy antigen receptor for chemokines), which capture interleukin (IL)-8, CCL5 and others, thereby limiting their bioavailability in tissues[26]. Further, chemoattractants are drained by the lymph fluid or blood. Lastly, chemokine degradation occurs through MMP2, MMP9 and other MMPs released by resident and infiltrate cells[46]. These proteases cleave many, if not all, chemokines, including CCL2, CCL7, CCL8, CCL13 and CXCL12, into either inactive or even antagonistic forms (reviewed in ref. 90). This function of MMPs downscales inflammatory infiltrates in a timely fashion and prevents tissue damage[46]. How these different mechanisms act together or synergize to limit interstitial leukocyte trafficking is unclear.

Exit control from tissues

Emigration from tissues and organs is confined to lymphocytes and DCs; it does not occur for granulocytes and macrophages. Exit of lymphocytes and DCs from lymph nodes provides peripheral tissues with activated effector cells for host defense[91]. In peripheral tissues, efflux delivers peripheral T cells and antigen-presenting cells (APCs) to the lymph node[1]. Efflux further limits and resolves an inflammatory response. In both lymph nodes and interstitial tissues, a monolayer of lymphatic endothelial cells (LECs) needs to be crossed, likely by a process of reverse diapedesis. In peripheral tissues, exit also may occur through gaps and blunt ends of terminal lymph vessels[92].

In the lymph node, exit routes are the paracortical and medullary sinuses. After 12–24 h of intranodal migration, T cells reach the sinus epithelium, which releases S1P into predominantly the afferent lymphatic vessel, thus likely creating a chemotactic gradient. In the absence of activation signals, passenger T and B cells express S1P receptors and require the S1P signal to cross the epithelium for exit[91]. However, once T cells receive TCR triggering and early activation signal by APCs leading to the upregulation of CD69, $S1P_1$ becomes downregulated for 2–3 d and the cells are prevented from exiting the lymph node[93]. Thus, down-modulation of a chemoattractant receptor that does not participate in maintaining baseline motility limits egress and confines mobile T cells to the lymph node until activation is complete.

In peripheral tissues, exit occurs into afferent lymph capillaries located parallel to blood vessels by a chemoattractant- and adhesion receptor–dependent process rather than by passive entry[94]. In T cells, experimental $S1P_1$ agonism causes migration arrest at the basal surface of the lymphatic endothelium through adhesion mediated by LFA-1–ICAM-1 and VLA4–VCAM-1 interactions[95]. Therefore, high S1P concentrations in inflamed tissue may support tissue retention of effector cells[95]. The egress of DCs from peripheral tissues is promoted mostly by chemokines. In epidermal Langerhans cells, signals induced by irritants or pathogens downregulate E-cadherin but upregulate CCR7 (refs. 92,96), which allows Langerhans cells to leave the epidermis, follow CCL21 constitutively produced by and decorating dermal LECs, and diapedese into lymphatic vessels of the upper dermis[96]. Thus, active reverse diapedesis rather than passive transport of lymphocytes and DCs mediates egress into afferent lymphatics[95]. At their basal surface LECs express macrophage mannose receptor and CLEVER-1, which have been implicated as counter-receptors for leukocyte adhesion to LECs and migration to lymph nodes[92,97,98]. The counter-receptors on leukocytes likely are not integrins, because integrin-deficient dermal DCs efficiently travel through afferent lymph vessels into lymph nodes[37]. In blood vessels of the liver sinus, monocytes use LFA-1 binding to ICAM-1 to migrate along the intraluminal endothelium of the vessel wall[99,100], but it remains to be shown where leukocytes use such haptokinetic mechanisms to move along the inner LEC walls and where passive transport occurs.

Migration during interstitial inflammation

Any acute or chronic inflammation in the tissue in response to damage, tumor growth or viral infection or in autoimmune disease and allograft rejection prompts tissue infiltration by effector cells, including neutrophils, monocytes, T cells and, in chronic states, B cells. Often, chronic inflammation causes *de novo*, tertiary lymphoid tissue formation, dependent upon a multistep developmental program of cell migration and positioning. Initially small and unstructured T cell infiltrates eventually segregate into a T cell area with DC networks, high endothelial venules and adjacent B cell follicles with germinal centers[101]. This process is initiated by lymphotoxin $\alpha_1\beta_2$ released from activated B and T cells, which activates stromal cells to release lymphoid homeostatic chemokines (CCL19, CCL21, CXCL12 and CXCL13)[101,102]. How the spatial separation into T and B cell areas occurs is unknown but, in reminiscence of secondary lymphoid organs, guidance and adhesion likely direct the temporary assembly of peripheral lymph follicles.

If persistent for weeks and months, chronic inflammation may eventually trigger the remodeling of tissue structures within and adjacent to connective tissues. In mouse models of rheumatoid arthritis, a progressive influx of granulocytes followed by T cells is observed preceding destruction[103]. Immigrated T cells of the $CD4^+$ memory phenotype reach the synovial membrane; secrete IL-2, interferon-γ and IL-17; and thereby activate monocytes, tissue macrophages, fibroblasts and the synoviocytes to produce ECM-degrading enzymes and fibrosis-inducing transforming growth factor-β, IL-13 and platelet-derived growth factor[104,105]. In later stages, B cells may infiltrate the synovium and form tertiary lymphoid tissue. Experimental arthritis in mouse models is reduced or abrogated by interfering with PI(3)K-γ (ref. 106) and LTB4 (ref. 107) signaling, which link interstitial trafficking, particularly of neutrophils, to disease outcome.

Migration in cytotoxic effector response

Cytotoxic T lymphocytes (CTLs) infiltrate solid tumors by moving along the tumor–matrix interface and along junctions between target cells (**Fig. 3d**)[108,109]. LFA-1–ICAM interactions have been implicated in CTLs crawling along tumor cell strands *in vitro*[110], but the *in vivo* mechanisms are unclear. In experimental tumors, activated CTLs reach their target site after a phase of movement through the adjacent stroma rather than from vessels inside the tumor[108]. Chemokines produced by tumor cells, tumor-associated macrophages[111] or fibroblasts[112,113], including CXCL12 (through CXCR4)[114,115], CXCL10 (CXCR3)[114,115] and CCL2 (CCR4)[116], are important in attracting effector T cells into the tumor tissue. Likewise, monocytes are recruited *de novo* and activated by CCL3, CCL4 and CCL5 released by tumor-infiltrating T cells[117]. Effector cells migrate through interstitial spaces and across the surface of stromal and tumor cells and scan for expressed cognate antigen (**Fig. 3d**). This 'search function' follows the rules of a persistent random walk, similarly to T cells in the lymph node cortex, as shown by *in vivo* microscopy[118,119]. With antigenic peptide present, fast scanning by CTLs converts to migration arrest and prolonged engagement with individual target cells in both virally infected or tumor cells[108,109,118,119]. An effector synapse polarizes TCR and LFA-1 to the target cell *in vitro*[119], which results in migration arrest until target cell apoptosis *in vivo*[108,109] and regression of the lesion[108,109,118,119]. Integrin $\alpha_E\beta_7$ engagement on CTLs supports polarized binding to target cells and enhances exocytosis of cytolytic granules[120], consistent with a function in adhesive costimulation. Other adhesion-promoting and costimulatory molecules, including Mac-1, LFA-1 and VLA-4, support $CD8^+$ effector T cell function in viral disease, but whether transendothelial migration, local positioning or both are favored remains unclear[121].

Tumors may also counteract the migration of infiltrating leukocytes. By unresolved mechanisms, stop signals can arrest the movement of tumor-infiltrating lymphocytes[122], and they represent a potential escape strategy. Further, the sequence of cells encountered before the target cells affects effector function. If nearby regulatory T cells suppress effector functions, target cell killing but not migration or conjugation may be impaired by a transforming growth factor-β–sensitive mechanism[123]. Thus, effector function is critically dependent on both efficient immigration of CTLs into the tumor and killing of it, and interference with either process supports target cell survival.

Migration during liver inflammation

Immune surveillance in the liver is provided by both resident and blood-borne 'passenger' lymphocytes and macrophages. In liver sinusoids, passenger lymphocytes scan endothelium and—without leaving the bloodstream, but rather through endothelial fenestrations— hepatocytes for antigenic peptide[124–126]. Likewise, natural killer (NK) T cells and monocytes crawl along and patrol the lumen of liver sinusoids[100] through binding of LFA-1 and chemokine receptor CX_3CR1 to their respective ligands ICAM-1 and CX3CL1 (also known as fractalkine) on liver endothelium[99]. During liver inflammation, such as viral hepatitis, NK, γδ T, NK T and $CD8^+$ effector cells become further recruited to cross

the endothelium, move between hepatocytes and diffusely infiltrate the liver[127,128]. This recruitment requires CCR5 and CXCR3 expression on leukocytes and CCL3, CCL4, CCL5, CXCL9 and CXCL10 produced by hepatocytes and sinus endothelial cells[128]. Recruitment of $CD8^+$ T cells into liver parenchyma and elimination of virally infected hepatocytes both depend upon CXCL9 and CXCL10 (ref. 129). Instead of recirculating, most infiltrate cells in the liver die within hours to days[127]; thus, little is known about exit mechanisms.

During chronic colonization of liver cells with intracellular bacteria, including *Mycobacterium tuberculosis*, migration and positioning of infiltrate cells leads to granuloma formation to locally confine and eliminate the infection[130]. Pathogen-infected macrophages recruit other macrophages and blood-derived monocytes to accumulate and form a dense cellular scaffold around the pathogen-infected region. This monocyte-derived convolute serves as track system for effector cells, including $CD4^+$, $CD8^+$, γδ and CD1-restricted T lymphocytes, which rapidly enter the macrophage network and engage in dynamic as well as sustained macrophage–T lymphocyte interactions[131]. Effector $CD4^+$ T lymphocytes secrete T_H1-type cytokines, such as interferon-γ, which activate macrophages and $CD8^+$ effector cells to eliminate intracellular pathogen and infected cells, respectively[130]. Thus, recruited macrophages form a structural barrier that hosts mobile T cells, conditioning the microenvironment with cytokines and effector molecules. Dynamic imaging data are not yet available for other inflammatory disorders, including glomerulonephritis, atherosclerosis, acute and chronic lung inflammation, and thyroiditis, which likely comprise similar recruitment and resolution mechanisms.

Therapeutic targeting of leukocyte trafficking in tissues

Many new anti-inflammatory drugs aim at interfering with transendothelial migration by antagonizing surface integrins, selectins or chemokines involved in leukocyte homing[8]. Because of its contribution in virtually every step of inflammation, interstitial leukocyte migration is another target function to modulate immune cell function in tissues (**Supplementary Table 1** online). In clinical use since the early twentieth century to blunt acute inflammation, colchicine prevents microtubule assembly by binding to tubulin, which inhibits chemotactic migration of neutrophils and monocytes into and within interstitial tissue[132]. More recently developed strategies to interfere with chemoattractant signaling show strong promise in reducing tissue damage in chronic inflammation[8]. Likewise, competitive inhibitors of the LTB4 receptor BLT1 reduce inflammation in models of rheumatoid arthritis and atherosclerosis[107,133]. FTY-720, an agonist of $S1P_1$, leads to short-term receptor activation followed by internalization and degradation and, thereby, suppresses S1P signaling. The FTY-720–induced inhibition of T and B cell egress from lymphoid and peripheral organs causes secondary lymphopenia and blunts T cell–dependent tissue inflammation[134]. Inhibition of PI(3)K-γ strongly reduces the infiltration of neutrophils and T cells in mouse models of chronic destructive inflammation, including rheumatoid arthritis and lupus-like syndrome[106,135].

To improve effector function, such as in established cancers, proinflammatory strategies aim at enhancing tissue infiltration by CTLs and cytolytic killing. Small molecule agonists of Toll-like receptors 7 and 8 activate cutaneous dendritic cells and macrophages. They release proinflammatory cytokines, including interferon-γ, tumor necrosis factor and the chemokines CCL3, CCL4 and CCL2, that attract CTLs and support CTL-mediated eradication of skin tumors in small-animal models and humans[136,137].

Conclusions

With increased kinetic information on different disease models, many interference strategies are likely to show effects on interstitial trafficking and signal exchange between cells. Because of their role in navigation control, chemokines and their downstream signaling pathways show promise as therapeutic targets, at both the vascular and interstitial tissue levels. Because diseases that often are not immediately fatal will be targeted, issues of safety will increase. To avoid unwanted chronic immunosuppression, fine-tuning of dosing and meaningful combination therapy will be needed. Targeting interstitial migration will need to aim at particular contexts and specific leukocyte subsets to modulate migration and positioning; this promises to complement interference with cell cycle progression (cytostatic drugs) or transendothelial migration (for example, integrin antagonists).

Interstitial leukocyte migration is regarded not only as means to change position from one place to another but as integral to immune cell surveillance and molecular recognition, including the search for APCs and target cells, the scanning of receptors expressed at the cell surfaces encountered and the accumulation of signals during migration and over time. Because of their amoeboid features, moving T cells act as bipolar sensory cells that integrate discrete signals over time until thresholds are reached and result in a cellular reaction, not unlike a mobile, position-changing neural cell. Whether myeloid cells collect signals in a similar, migratory manner is not known. In conclusion, live-cell microscopy has shifted our conceptions of immune processes from an abstract, imagined appreciation of leukocyte trafficking into a visual and more detailed understanding of how, when, where and in which sequence immune cells get together, exchange information and eliminate pathogens and tumor cells.

Note: Supplementary information is available on the Nature Immunology website.

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