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T cell migration in intact lymph nodes *in vivo*

Marcia A Munoz[1,4], Maté Biro[1,2,4] and Wolfgang Weninger[1,2,3]

In the lymph node, T cells migrate rapidly and with striking versatility in a continuous scan for antigen presenting dendritic cells. The scanning process is greatly facilitated by the lymph node structure and composition. *In vivo* imaging has been instrumental in deciphering the spatiotemporal dynamics of intranodal T cell migration in both health and disease. Here we review recent developments in uncovering the migration modes employed by T cells in the lymph node, the underlying molecular mechanisms, and the scanning strategies utilised by T cells to ensure a timely response to antigenic stimuli.

Addresses
[1] Centenary Institute of Cancer Medicine and Cell Biology, Immune Imaging Program, Locked Bag 6, Newtown, NSW 2042, Australia
[2] Sydney Medical School, The University of Sydney, Sydney, NSW 2006, Australia
[3] Department of Dermatology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia
[4] These authors contributed equally to this work.

Corresponding author: Weninger, Wolfgang (w.weninger@centenary.org.au)

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Introduction

The remarkable migratory skills of T lymphocytes are central to the immunosurveillance of tissues and the development of adaptive immunity against infection and cancer. Antigen-inexperienced 'naïve' T cells are continuously trafficking between the vasculature and lymph nodes (LNs) in search of cognate antigen [1] The LN, a multifunctional and compartmentalised organ, brings antigen-loaded dendritic cells (DC) and a large number of scanning T cells in proximity. Upon antigen encounter on DCs, naïve T cells can be primed into an 'effector' state, ready to exert their cytotoxic, helper or regulatory function in pathogen-laden or neoplastic tissues (reviewed in [2]). Both naïve and effector T cells possess a cell-intrinsic aptitude for tissue scanning, yet in the crowded landscape of the LN, their migration is in addition governed by an array of extrinsic biomechanical cues. Accordingly, the architecture and composition of the LN collectively shape a guidance structure that is fundamental for optimal T cell scanning. Recent advances in intravital multiphoton microscopy (IVMM) modalities have allowed unprecedented insight into the complex migratory behaviour of T cells within the lymphoid microenvironment. In this review, we use T cell locomotion in the LN as a paradigm to understanding *in vivo* leukocyte motility. We discuss the cellular and molecular mechanisms that govern their motility in the LN both in the steady state and during inflammation.

Migration mode of T cells *in vivo*

T cells are highly versatile migrators and can effectively navigate almost any tissue of the body. As other leukocytes, T cells migrate using an 'amoeboid' mode of locomotion in three-dimensional *in vivo* environments [3]. The amoeboid migration of T cells is characterised by a rounded yet polarised morphology. Frequent and rapid extensions of pseudopodia at the leading edge are driven by the polymerisation of filamentous actin (F-actin) that extends the plasma membrane in the direction of migration (Figure 1) [4]. In contrast, stable F-actin networks rich in myosin motors characterise the uropod, a lagging tail that undergoes little overall deformation [5]. T cells can rapidly alternate between adhesion-dependent and adhesion-independent motility in order to adapt to the varying tissue architecture and molecular composition of their surrounding. In non-adhesive environments, leading edge pseudopodia are 'wedged' into confined spaces in the extracellular matrix (ECM), which, coupled with actomyosin contraction of the rear, allows for a concerted translocation of the cell [4,6,7]. During adhesion-driven migration, nascent integrin-mediated attachments in the extended pseudopodia anchor the cell to the substrate. The retrograde flow of treadmilling actin filaments coupled to the ECM subsequently drives forward displacement of the cell [8]. The contractile uropod is also adherent, albeit only loosely, and actomyosin-mediated forces help detach mature adhesion sites and propel the cell body forward (Figure 1). This distal contractility is however partly dispensable in the LN, as shown by both pharmacological inhibition [7] and conditional knockout [9] of myosin function, upon which T cells migrated suboptimally with an elongated shape, at slower speeds and with less directionality. Surprisingly, it was found that in T cells, actomyosin contractility is mainly required for de-adhesion from the ECM rather than to facilitate the passage of the rigid nucleus through the dense lattices found in the LN [7], in stark contrast to what has been observed for DCs [10]. Blocking antibodies and genetic knockout models demonstrated that adhesion, mainly mediated *via* the integrin LFA-1 and its ligand ICAM-1, is not absolutely required for T cell motility, but

GLOSSARY BOX 1: Modes of chemokine-driven migration
Chemokinesis: non-directed migration in response to soluble chemokines.
Haptokinesis: non-directed motility supported by immobilised chemokines.
Chemotaxis: guided motility following a soluble chemokine gradient.
Haptotaxis: directed adhesive migration following a gradient of substrate-bound chemokines. Although available *in vivo* evidence strongly supports that T cells employ haptotaxis within the LN, haptotactic guidance remains to be formally demonstrated.

is essential for optimal intranodal scanning [6,7,11,12•,13]. Consistently, targeted deletion of the LFA-1 adaptor protein Kindlin-3 did not fully abrogate T cell migration [14]. LFA-1 was however shown to promote T cell retention in the LN and thus prevents premature termination of scanning [12•]. Taken together, T cells migrate predominantly in a weakly adhesive and contractile mode where integrin activity must be tightly regulated to generate enough traction without compromising speed, and where posterior actomyosin contractility facilitates propulsion of the uropod. However, T cells are endowed with sufficient redundancy to overcome the lack of both actomyosin contractility and integrin-mediated adhesiveness during migration.

Molecular mechanisms of T cell migration in the LN

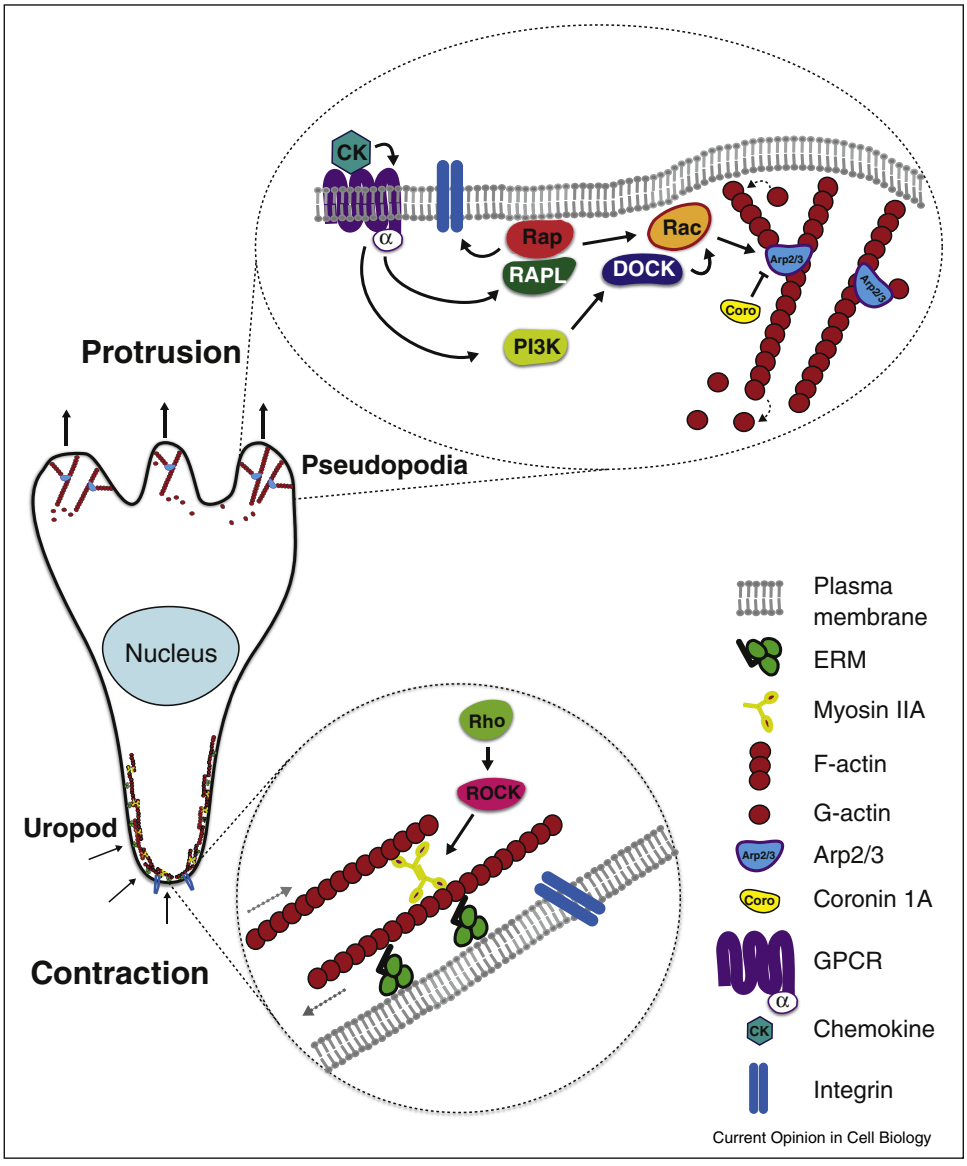
Intranodal T cell migration is dependent on signalling mediated by G protein-coupled receptors (GPCRs), and their Giα subunits (Figure 1), as shown by the reduction in speed and displacement observed following pertussis toxin (PTX, a Giα-specific inhibitor) treatment [15–17]. Binding of the GPCR CCR7 by its ligands CCL19 and CCL21 stimulates the migratory behaviour of T cells within the LN paracortex, principally by promoting high-speed motility [15,18•]. However, while the absence of CCR7 signalling resulted in only a partial reduction in motility, inhibition of Giα by PTX induced a more pronounced migratory defect [15,16]. Investigation of alternative chemokine axes such as CXCR4/CXCL12 and lipid mediators have revealed only partial contributions to intranodal T cell motility (reviewed in [19]), indicating the involvement of as yet undiscovered GPCRs.

Ligand binding to GPCRs triggers signalling cascades that govern cytoskeletal dynamics, polarity establishment and motility *via* GTPases of the Rho or Rap families (reviewed in [20,21]). In T cells, the Rho-GTPase Rac polarises to the leading edge, where it stimulates Arp2/3 complex-driven branched actin network formation and the expansion of pseudopodia (Figure 1). Abrogation of Rac function, either *via* double knockout of Rac1 and Rac2 [22•] or *via* deletion of its upstream activator DOCK2 [23•], led to acute migratory defects in the LN. Rac-deficiency had a more severe effect than chemokine depletion alone [22•], highlighting the central role of Rac-mediated signalling downstream of GPCRs and an additional cell-intrinsic role in T cell migration. Rac1 and Rac2 were moreover shown to be functionally redundant, as single knockout of either isoform did not significantly alter the migration characteristics of T cells [22•]. Downstream of Rac, the ezrin-radixin-moesin (ERM) proteins, concentrated in the uropod, link the plasma membrane to the actomyosin cortex and modulate T cell polarisation and migration through regulation of cortical tension and by restricting protrusion formation to the leading edge [24,25] (Figure 1). Expression of a constitutively active form of ezrin in T cells resulted in a limited ability to undergo shape changes, restricted lamellopodia formation and a marked defect in intranodal migration [25]. Another regulator of actin remodelling is the actin binding protein Coronin 1A, an inhibitor of Arp2/3 activity. A mutation in Coronin 1A that prevents its recruitment to the leading edge results in dysmorphic and often oversized anterior protrusions in T cells. Strikingly, Coronin 1A mutant cells displayed slower and less directional intranodal migration than their wildtype counterparts [26]. These studies reveal that both excessive and insufficient Rac-activity and Arp2/3-activity at the leading edge lead to defective motility, underlining the tight spatiotemporal regulation of F-actin dynamics in migrating T cells.

The GTPase Rap1 and its effectors RAPL and Mst1 kinase are also necessary for effective intranodal T cell migration [17]. Rap1 initiates chemokine-induced cell polarisation by mediating the activation of Cdc42 and Rac [27]. *In vivo* imaging studies revealed that RAPL [17] and Mst1 [28•] knockout T cells failed to establish a polarised shape and displayed a significant reduction in speed and displacement within the LN, comparable to GPCR inhibition but more severe than integrin blockade. These studies demonstrate that cytoskeletal and morphological polarisation are essential for intranodal T cell migration.

GPCRs and T cell receptors (TCR) also signal to downstream phosphoinositide 3-kinase (PI3K) [29], an enzyme that catalyses the phosphoinositide conversion required for the plasma membrane recruitment and assembly of signalling complexes such as the Rac activator DOCK2 [21,29]. In contrast to the better-known function of PI3K signalling in other leukocytes [30], the contribution of PI3K to intranodal T cell migration remains incompletely understood and somewhat controversial. In one study, pharmacological PI3K inhibition with Wortmannin and genetic deletion resulted in slower motility with no effects on other migratory parameters [31]. On the other hand, T cells lacking the PI3Kγ isoform, displayed reduced directionality but no observable differences in speed [23•]. Yet a third *in vivo* imaging study in LN slices reported that PI3K did not contribute to intranodal T cell motility, as shown by Wortmannin treatment [32]. The reasons for these conflicting reports could be attributable

Figure 1



Signalling pathways in intranodal T cell migration *in vivo*. Schematic representation of a polarised T cell during migration. Migrating T cells are characterised by a protruding leading edge and a contractile lagging uropod. **(Top insert)** Chemokine binding to GPCRs in the pseudopodia triggers localised Rap/RAPL signalling, Rac activation, and the formation of nascent integrin adhesion sites. In parallel, PI3K contributes to Rac activation *via* DOCK2. Rac drives Arp2/3-mediated branched actin network formation, membrane protrusion and pseudopod extension. Coronin 1A binds to and inhibits Arp2/3-driven actin polymerisation. **(Bottom insert)** Enrichment of activated ERM (ezrin-radixin-moesin) proteins in the uropod reinforce membrane-cortex attachments to increase cortical stability and prevent posterior protrusions. Rho, *via* its effector kinase ROCK, activates myosin IIA-mediated actomyosin contraction. The contractile forces generated serve to disengage the integrin clutch linking the cortex to the extracellular environment during adhesion (dotted arrows), and propel the trailing end forward.

to differences in experimental models, T cell subsets with different requirements for PI3K signalling, and functional redundancy among PI3K isoforms [29].

T cell scanning of the LN

IVMM observations have revealed that T cells move constitutively, rapidly and randomly throughout the paracortex with variable speeds that average ~10–12 μm/min (reviewed in [33]). T cells migrate in a 'stop-and-go' pattern characterised by sudden bursts of high-speed directional locomotion ('runs'), followed by short migratory arrests or slow confined movement during which T cells interact with the stroma and other leukocytes (Figure 2b) [34,35]. The onset of such transient arrests and slower motility intervals are accompanied by intermittent, low amplitude intracellular Ca^{2+} spikes in a

subset of T cells in the LN [32,36]. It is becoming increasingly clear that the magnitude and duration of Ca^{2+} bursts directly influence the basal speed of T cell motility in the LN. However, the mechanisms linking Ca^{2+} signalling to cytoskeletal remodelling and ensuing adjustments to T cell locomotion are still incompletely understood (reviewed in [37]). It remains to be demonstrated whether the spatiotemporal dynamics of intracellular Ca^{2+} flickers govern T cell velocity and steering in the LN, as has recently been shown in other cell types *in vitro* (reviewed in [38]). T cells maintain their amoeboid front-rear polarisation while engaging in brief asymmetric contacts with DCs [39]. It is still unclear whether this stop-and-go behaviour is the result of obstacle collision or due to intrinsic cellular cues, or a combination of both [40]. The frequency and duration of probing interactions between T cells and other immune cells greatly influence their overall scanning speed. In the absence of cognate antigen, the fast basal motility of T cells together with actively probing DCs is estimated to result in a contact frequency of ~100 different DCs/hour for a single scanning T cell [41]. Initial IVMM studies proposed a cell-intrinsic and autonomous behaviour that fitted the 'random walk' model of particle diffusion (reviewed in [42]). However, subsequent imaging studies revealed that lymphocyte motility *in vivo* is highly dependent upon local environmental cues, giving way to the concept of guided random migration [18•,43••,44••].

Guidance of T cell migration in the LN

The paracortex is constituted by a scaffold of ECM conduits ensheathed by fibroblastic reticular cells (FRC) (reviewed in [45]). This structure is interspersed with DCs and forms the FRC network, an intricate transportation grid that supports efficient T cell migration and scanning, as well as entry into the paracortex *via* high endothelial venules (HEV) (Figure 2a) [43••,46,47]. FRCs produce the chemokines CCL19 and CCL21 (reviewed in [19]), which contribute to the spatial segregation of the cortex into T and B cell zones by supporting T cell migration only within the paracortex [15,18•] and guiding the penetration of lymph-derived T cells into the deep T cell zone [48].

T cells migrate differently in different areas of the LN. For example, T cells move slower in the medulla and the subcapsular region than in the paracortex (Figure 2) [18•,23•]. Such variety in migratory behaviours is likely due to differences in the distribution and availability of chemokinetic/haptokinetic stimuli (Glossary Box 1) [15,18•], and structural and physiological particularities of each LN compartment [16]. Moreover, $CD4^+$ T cells recirculate more rapidly and spend shorter periods in each LN (~12 h) than their $CD8^+$ counterparts (~21 h) [49•]. Contrary to $CD8^+$ T cells, $CD4^+$ T cells rely on the presence of major histocompatibility complex (MHC) molecules on the surface of DCs to maintain an optimal scanning mode [49•].

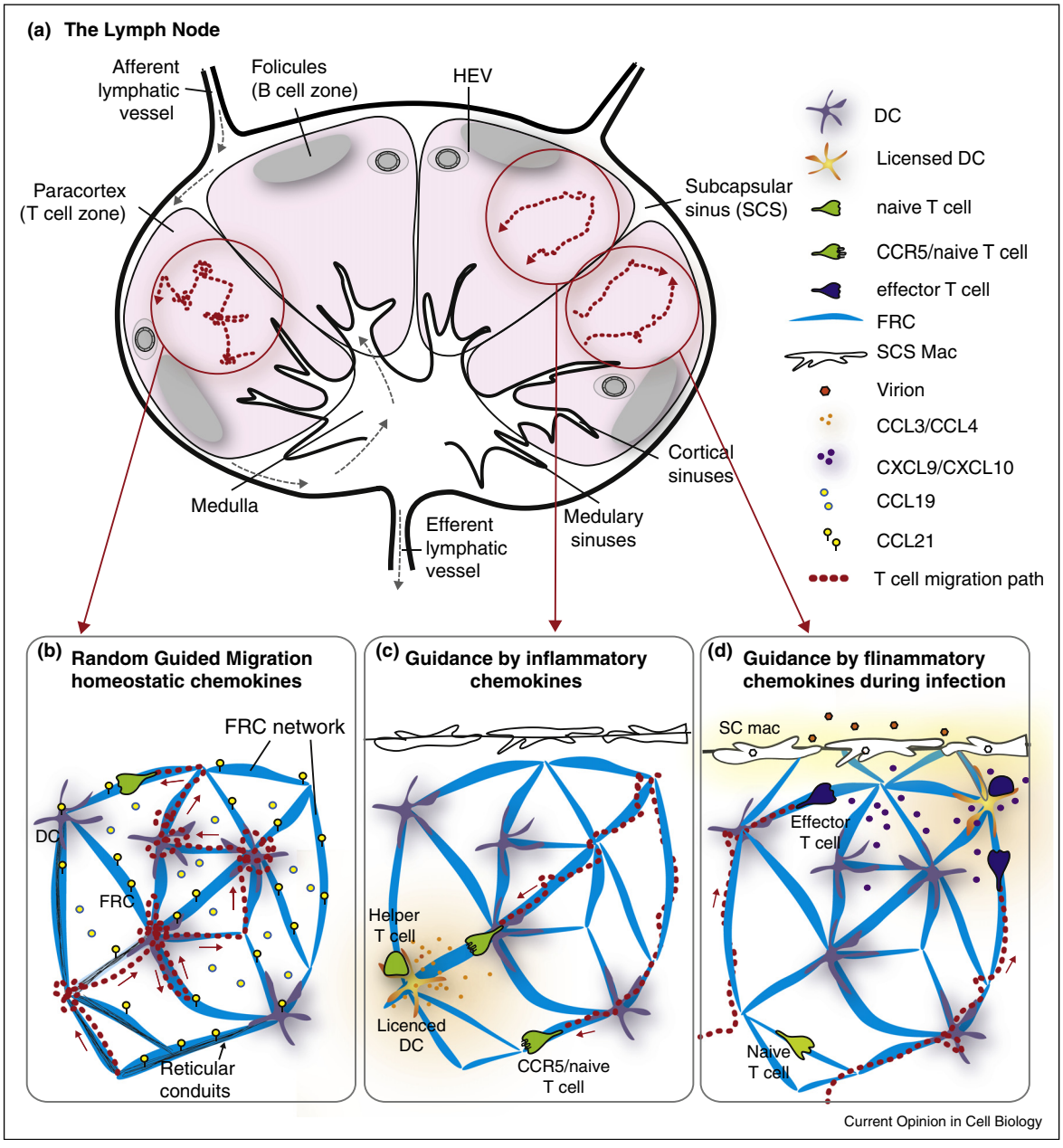
Scanning in the presence of cognate antigen

Upon antigenic encounter, T cells alter their movement. They initially display restricted motility, followed by migratory arrest and stable engagements, and finally a phase of dissociation and resumption of migration [50,51]. Reduction in motility and arrest are triggered by a 'stop-signal', which enables the thorough probing and cellular engagement with antigen-loaded DCs [52]. This antigen-mediated stop signal is associated with higher frequency and amplitude Ca^{2+} spiking than what has been observed during homeostatic T cell scanning [36]. The presence of suppressive regulatory T cells interferes with the stop-signal and promotes motility upon cognate encounter, mainly by hampering antigen recognition [53–57]. In addition, the cytotoxic lymphocyte-associated antigen-4 (CTLA-4) and the program-death receptor 1 (PD-1) are negative regulators of T cell activation that act partly by blocking the stop-signal and promoting cell migration in the presence of antigen [58•,59•]. However, while PD-1 blockade in T cells resulted in the restoration of the stop-signal and reduced displacement, CTLA-4 inhibition did not [59•]. Comparison of conventional and regulatory T cells revealed that the latter were partially resistant to CTLA-4-mediated perturbation of the stop-signal [60], highlighting the heterogeneity of signals that govern the migratory behaviour of different T cell subsets.

T cell mobilisation to specific LN microenvironments during priming provides an additional layer of regulation, contributing to phenotypic differentiation and functional outcome [61,62•,63•]. The CCR5 ligands CCL3 and CCL4, and the CXCR3 ligands CXCL9, CXCL10 and CXCL11, are produced in response to infection or injury and promote the recruitment of immune mediators to the site of inflammation (reviewed in [63•]). These chemokines have been shown to facilitate cognate encounter by biasing T cell mobilisation towards antigen-loaded DCs in the LN [44••,61,64,65]. Activated T cells engage antigen-presenting DCs, 'licensing' them to produce CCL3 and CCL4, thus attracting more naïve T cells, which during inflammation upregulate CCR5 (Figure 2c) [44••]. Similarly, CXCR3 mediates intranodal recruitment of T cells to the site of antigen accumulation (interfollicular and medullary zones) for effective activation by CXCL10-producing DCs [61,62•] (Figure 2d). CXCR3-mediated mobilisation is more efficient for memory than for naïve T cells [62•].

Recent IVMM studies in infectious models in the LN revealed a cellular immune network consisting of a wide variety of leukocytes including $CD8^+$ T cells, strategically positioned near the periphery of the LN and pathogen-sensing phagocytes [63•]. Upon infectious challenge, effector memory $CD8^+$ T cells are rapidly recruited from

Figure 2



Lymph node structure and migratory behaviour of T cells within the T cell zone. **(a)** Simplified diagram of the lymph node structure depicting the cortex (pink) encased by the capsule. The cortex is subdivided into paracortex and B cell follicles. Each paracortical region is infiltrated by a high endothelial venule (HEV). The subcapsular sinus (SCS), a space separating the capsule from the cortical parenchyma, collects the lymph from afferent lymphatics and delivers it to the medullary region and the efferent lymph vessel. The medulla is rich in lymphatic endothelial structures called medullary sinuses that penetrate the paracortex to become cortical sinuses. Grey arrows illustrate the flow of the lymphatic fluid within the lymph node. **(b)** The paracortex is irrigated with ultrafiltrate lymph by an intricate scaffold of reticular conduits composed mainly of collagen fibres (clusters of black lines) ensheathed by fibroblastic reticular cells (FRC, blue) that collectively form the FRC network. DCs are incorporated into the FRC network providing an optimal transportation and scanning platform that 'guide' random basal T cell migration. T cell motility in the steady-state is stimulated by soluble CCL19 and immobilised CCL21. The red dashed line indicates the random stop-and-go migratory behaviour of T cells. **(c)** T cells 'license' an antigen-loaded DC to produce CCL3 and CCL4 and promote the recruitment of other CCR5-expressing naïve T cells. Dashed line depicts the directional migratory behaviour of T cells in response to inflammatory chemokines. **(d)** Lymph-borne infectious particles are captured by strategically positioned SCS macrophages, which in turn trigger an inflammatory response that results in the fast and directional recruitment of effector T cells (dashed line) [4].

the deep paracortex towards the periphery in a CXCR3-dependent manner (Figure 2d) [62•,66•,67]. $CD8^+$ T cells travel to the site of pathogen accumulation where they become activated [66•,68–70]. Furthermore, engagement of memory $CD8^+$ T cells with DCs triggers the release of IFNγ and CXCL9, promoting further mobilisation and activation in a self-propagating feedback loop [66•]. In summary, chemokine guidance greatly increases the odds of antigenic encounters by surveying T cells.

Concluding remarks

Significant progress has been made in the elucidation of the mechanisms underlying T cell motility in the LN, notably owing to advances in intravital imaging modalities. Recent studies have revealed that optimal LN scanning results from a combination of the intrinsic migratory versatility of T cells, the local chemokine milieu and the intricate microarchitecture of the LN. The remarkable ability of T lymphocytes to switch from one migration mode to another is underpinned by a complex cross-talk of signalling pathways, of which the small GTPases of the Rho and Rap family are central. Targeting T cell navigation to curb pathological immune disorders or to boost the host's anti-tumour response could constitute an attractive therapeutic target, as indicated by emerging treatments based on the inhibition of integrins [71], PI3K [72], Rho-GTPases [73], CTL-A and PD-1 [74], all key regulators of T cell migration. The next challenge, however, will be the complete mapping of these signalling cascades, and a better understanding of the variations in the regulation and function of these pathways among different T cell subsets.

Conflict of interest

The authors have no conflict of interest to declare.

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