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# The spatiotemporal control of effector T cell migration

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## Abstract

Effector T cells leave the lymph nodes armed with specialized functional attributes. Their antigenic targets may be located anywhere in the body, posing the ultimate challenge; how to efficiently identify the target tissue, navigate through a complex tissue matrix and ultimately locate the immunological insult. Recent advances in real time in situ imaging of effector T cell migratory behaviour have revealed a great degree of mechanistic plasticity that enables effector T cells to push and squeeze their way through inflamed tissues. This process is shaped by an array of 'stop' and 'go' guidance signals including target antigens, chemokines, integrin ligands, and the mechanical cues of the inflamed microenvironment. Effector T cells must sense and interpret these competing signals to correctly position themselves to mediate their effector functions for complete and durable responses in infectious disease and malignancy. Tuning T cell migration therapeutically will require a new understanding of this complex decision-making process.

## Introduction

The rapid and targeted response of the immune system to tissue damage is reliant on the incredibly nimble movement of leukocytes between and within tissues. Tissue-specific mobilization depends on the ability of leukocytes to sense directional signals from specialized niches and to quickly respond and adapt to environmental cues and tissue landscapes remodeled by infection and inflammation. T cells surveying tissues move fast, but have the capacity to rapidly arrest upon antigen encounter. This ability for quick decision-making with respect to locomotion has captivated cell biologists, biophysicists and bioengineers alike who have used immune cells as a powerful tool to understand the mechanics of movement within 3D tissues. For immunologists, understanding the regulation

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of leukocyte migration is central to our ability to control immune function in health and disease.

T cell activation and function is dependent on the efficient scanning of inflamed tissues for the identification of cellular targets. Those targets are often innate immune cells capable of presenting cognate antigen to $CD4^+$ and $CD8^+$ T cells and non-haematopoietic targets in the form of infected, damaged or malignant cells. Optimal strategies to 'find' such targets will differ, depending on the location and distribution of targets as well as on the activation state of the T cell (that is, whether a T cell is naïve, effector or memory). Naïve T cells survey the extensive reticular cell network of the lymph node for rare ligand-bearing antigen presenting cells (APCs) during the initiation of an immune response. Meanwhile, effector T cells enter inflamed tissues many days after the initial insult and are met by a tissue microenvironment reshaped by inflammatory cytokines and innate cell recruitment. Within this context, effector T cells must integrate a plethora of extrinsic signals to optimize encounter with APCs for reactivation, and position themselves for precise delivery of effector function, while limiting collateral tissue damage. Since the discovery of lymphocyte recirculation by Gowans, significant progress has been made in understanding the migratory capacity of T cells between tissues and within the confines of the lymph node[1,2]. This Review focuses on the dynamic behaviour of effector T cells once they enter an inflamed site. We discuss the complexity of T cell movement within inflamed tissues and highlight the unique navigational challenges they face. Understanding spatiotemporal regulation of T cell migration will lead to novel therapies aimed at inhibiting the dysregulated autoimmune and allergic conditions as well as augmenting the host response to tumours, infectious agents and vaccines.

## A matter of scale

The use of intravital imaging and new super-resolution imaging modalities has provided an unprecedented window into the leukocyte–tissue interface and has revealed both the requirement, and lack-thereof, of numerous migration cues including chemokines, integrin ligands, extracellular matrix (ECM) components and lipids. These often contradictory findings reflect context-dependent differences in the tissue and inflammatory milieu, and limitations imposed by the level of experimental analysis. Direct imaging of individual immune cell types in their native context provides the most accurate spatiotemporal assessment of immune effector functions, but the complexity of the milieu makes it challenging to parse out individual molecular contributions. Single-cell high-resolution imaging in controlled fabricated settings can define molecular dynamics of movement but such studies lack biological complexity, often testing one or two parameters at a time. Thus, there is an inherent inverse correlation between biological complexity and molecular resolution and a pressing need to bridge these levels to fully understand how T cells position themselves for action (Fig 1). Despite these divisions of scale, there is growing consensus that immune cell migration needs to remain plastic in the face of microanatomical changes in tissue terrain, cellular composition and 'target' distribution[3–7]

## Basic mechanics of 3D movement

Before we discuss context-specific requirements for T cell migration, we will briefly review the basic parameters for cell movement in 3D[8,9]. T cells migrating in 3D tissues move fast (10–15μm/min), often in a random fashion, and are confined by cellular networks embedded in ECM. Such locomotion requires cell polarization and the transmission of force between the cell and its environment carried out by adhesion molecule transmembrane force coupling (usually integrin-based) or friction with the surface, resulting in forward actin protrusions and actomyosin contraction (Box 1)[10]. The efficiency of migration is tuned by the degree of force generated and the extent of traction provided. Classically, modes of migration have been separated into two main forms; slow, high adhesive, movement (mesenchymal, highlighted by fibroblasts and cancer cells) and fast, low adhesive, movement (amoeboid, exemplified by immune cells). In practice, modes of migration are fluid, with a seemingly seamless transition between adhesion and non-adhesion-dependent modes[11]. Movement is therefore the sum of numerous input signals supplied by different signalling pathways in a spatiotemporal fashion (Fig 2).

Unlike fibroblasts and tumour cells that use matrix metalloproteinases to breakdown matrix and forge their own path through tissues, most leukocytes do not utilize proteolysis for interstitial migration and therefore their migratory path is constrained by the size of natural channels and pores within the ECM scaffold[12]. Activated T cells moving in 3D collagen matrices make frequent directional changes in regions of narrow space[13], which may explain the random migration patterns of effector T cells in infected tissues as diverse as the brain, lung, liver and skin[7,14–17]. High-resolution analysis in microfabricated 3D collagen gels suggests that when faced with a directional ‘choice’, T cells distinguish between differently sized pores in the matrix and preferentially migrate along the path of least resistance[13,18]. Rite of passage will be shaped by the interfibrillar space and the relative deformability of both the matrix fibres and the cell (Box 2)[9]. The degree of matrix resistance will depend on tissue-specific specialization of the matrix structure and context-dependent inflammatory mediators. T cells are extremely soft cells, with the rigidity of human T cells being lower than that of all myeloid cells tested[19]. Optimal pore size is regulated by the size and deformability of the cell’s nucleus, which is often 2 to 10 times stiffer than the cell body[20,21]. In fact, leukocytes migrate with the nucleus at the front of the cell where it appears to serve as a mechanical gauge, with the cell opting for a direction that best fits the ability of the nucleus to protrude into the space[18]. Neutrophils are particularly adept at quick passage through dense tissues aided by an unusually flexible nucleus that lacks lamin A/C intermediate filaments that usually form a rigid shell underneath the nuclear membrane[22–24]. Dendritic cells (DCs) optimize nucleus deformation by generating a dense and dynamic perinuclear actin network[25]. A recent study in T cells highlighted the role of formin-like 1 (FMNL1) in maneuvering of the nucleus through tight spaces[26]. Upregulation of FMNL1 has been linked to effector T cell trafficking in disease[27,28] and may suggest that induced changes in the relative deformability of the nuclear can influence T cell movement within inflamed tissues.

In vivo, the size of the gaps in the matrix are dynamically regulated by the inflammatory milieu that can narrow or widen the spacing[29]. Migration in dense 3D tissue

microenvironments has been shown to be largely adhesion (or integrin) independent[30], (see exception below), utilizing frictional forces to 'chimney' between matrix fibres[8]. A recent *in vitro* study suggests that in tight confined spaces the topography of the microenvironment itself can support locomotion in the absence of receptor-mediated adhesion. When confined in 3D, T cells failed to migrate on a smooth surface in the absence of integrins, however provision of serrations to the surface was sufficient to support T cell locomotion[31]. The retrograde flow of actin followed the texture of the substrate and created sufficient shear forces to propel the cell forwards. A new *in vivo* study of tissue resident memory T ($T_{RM}$) cells in the salivary glands supports the idea of integrin and chemokine-independent friction-based motility with macrophages providing the necessary topographical surface to support tissue surveillance[32]. This topographical model would allow T cells to autonomously move seamlessly through varying tissue landscapes regardless of the provision of extrinsic cues.

## Guidance cues and their distribution

The 3D movement of leukocytes within inflamed tissues is controlled by the organization of the tissue ECM and the availability and distribution of chemotactic and adhesive signals. As illustrated in Figure 2, the balance between these guidance cues will dictate the most efficient mode of migration. Indeed, a recent study of effector T cell homing via afferent lymphatics nicely demonstrates the interplay between chemokines, integrins and mechanical constraints[33]. Individual guidance cues and their molecular mechanism of action have been reviewed in detail elsewhere[29,34–37]. Here we will discuss the 'presentation' of these cues within the tissue and how they may modulate effector T cell migration at multiple stages; from exiting the blood stream and traversing the inflamed tissue to identifying antigen-bearing or infected targets (Fig 3).

### Chemokine presentation in tissues.

Chemokines play a central role in the determination of tissue-specificity and selectivity during T cell recruitment[34]. They direct migration by binding to G protein-coupled receptors (GPCRs) that initiate signalling cascades that can activate integrins and regulate remodelling of the actin cytoskeleton[35]. Initial T cell entry into inflamed tissues, or trans-endothelial migration (TEM), is guided by immobilized chemokines on surfaces of the endothelial cells and the glycocalyx[36,38] . In addition, intra-endothelial chemokines stored in intracellular vesicles of the endothelial cells can guide TEM[36,38]. Immobilization is achieved by binding to sulphonated glycosaminoglycans (GAGs), such as heparan sulphate, that are covalently attached to endothelial cell-associated matrix. GAGs are negatively charged linear polysaccharides with incredible diversity due to differences in their length, composition and patterns of acetylation and sulphation[39,40]. Their ability to interact with other charged molecules such as chemokines makes them critical regulators of chemoattactant availability and stability that shapes the nature of the chemokine gradient. A steep gradient of heparan sulphate between the apical and basolateral sides of endothelial cells provides a mechanism for patterning of haptotactic gradients of the chemokines released by endothelial cells and pericytes, which can drive directional leukocyte migration during TEM[41]. It should be noted that immune cells can enter some visceral organs via a non-vascular route[42,43] and will likely be regulated by distinct mechanisms of tissue-entry.

Within the tissue, there are a variety of GAGs that are mostly attached to core proteins of the ECM (heparin/ heparin sulphate, chondroitin sulphate/dermatan sulphate, keratan sulphate, and hyaluronic acid)[44], although hyaluronan can also be found 'free' or non-ECM bound[44]. The levels of expression of these GAGs are dramatically increased in inflamed tissues, chronic infection and autoimmunity. Mutations in chemokines that reduce their capacity for GAG-binding abrogate leukocyte migration, highlighting the importance of chemokine–GAG interactions[45,46]. Chemokines likely exist in dynamic equilibrium between soluble and GAG-bound forms and will vary in their oligomerization states depending on the specific GAG interactions[39,47]. Growing evidence now suggests that chemokine signalling residues that mediate receptor interactions are also involved in GAG binding, indicating that GAG-bound chemokines may be unable to directly activate receptors of migrating leukocytes[48]. Instead, chemokine-GAG binding may function as a local depot in inflamed tissues, serving to preserve the microanatomical position and longevity of chemokines that would otherwise readily diffuse away from the induction site. How the release of chemokines from GAGs is regulated to inform cell migration is not well understood. During inflammation, GAGs can be released from their proteoglycan backbone by enzymes like heparanase, which may alter the ratio of bound to free chemokine. The newly released soluble chemokines may contribute to the local fine-tuning of chemotaxis to modulate cellular trafficking[49], although this has yet to be demonstrated for leukocyte migration.

### Spatially distinct integrin ligands and functionally distinct integrin expression.

Lymphocytes express numerous integrins that mediate cell–cell and cell–matrix interactions[50]. Integrins LFA1 ($\alpha_L\beta_1$) and VLA4 ($\alpha_4\beta_1$) on effector T cells mediate TEM by binding to ICAM1 and VCAM1, respectively, that are upregulated on the vascular endothelium of inflamed tissues[36]. Once within the tissue, expression of matrix-binding integrins can facilitate interaction with the ECM and control migration and positioning at the inflamed site (see ECM section below). Specificity is determined by the α and β subunit pairings, and these integrins bind numerous ECM proteins such as laminins ($\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_V\beta_1/\beta_3$, $\alpha_6\beta_1$, $\alpha_7\beta_1$), fibrillar collagens ($\alpha_2\beta_1$, $\alpha_3\beta_1$), Arg-Gly-Asp (RGD) motifs contained in many ECM proteins like fibronectin and laminin ($\alpha_5\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_{IIb}\beta_3$) and proteins expressed on the tissue barriers like E-cadherin ($\alpha_E\beta_7$). Integrin binding avidity can be enhanced by extracellular cues such as chemokine-dependent conformational change and increased ligand-induced clustering[51] (Box 1). Large integrin-based focal adhesions appear incompatible with the rapid amoeboid migration displayed by T cells, and is supported by studies showing integrin-independent leukocyte movement in 3D tissues[30,31]. It is worth noting here that, although possible to move in the absence of integrins, it is unlikely both in normal physiology and pathological conditions that actively migrating leukocytes are not constantly exposed to integrin ligands. How T cells tune their sensitivity to such integrin binding activity in the face of overwhelming ligand and chemokine activation cues is not well understood[52]. Actively migrating T cells likely form small, highly dynamic focal adhesions that provide just enough friction force to allow cells to rapidly shift back and forth between integrin mediated and non-integrin mediated modes of cell migration to optimize speed and tissue coverage[11].

If not essential for interstitial migration per se, integrin ligands provide important localization cues. Crossing the basement membrane is a rate limiting step for leukocyte tissue entry[53,54]. The composition of the basement membrane varies along the length of post-capillary venules and can dictate tissue entry[55], with sites of low laminin $\alpha_5$ being preferred sites of $CD4^+$ T cell entry into the CNS, via T cell expression of $\alpha_6\beta_1$ integrin[56,57]. The functional importance of T cell interactions (via $\alpha_6\beta_1$ and $\alpha_V\beta_1$) with distinct laminins was recently highlighted in the mouse experimental autoimmune encephalomyelitis model of multiple sclerosis[58]. Loss of laminin 511 resulted in enhanced disease severity, that was in part explained by T cell high-affinity integrin interactions with laminin 511 that appears may limit TEM, and an interesting migration-independent role for laminin-511 in limiting the differentiation of pathogenic Th17 cells[58]. The 'looser' fibrillar network of the interstitial matrix is composed of fibrillar type I or III collagen which make up 90% of proteins in tissues, providing physical stability and a scaffold for other ECM proteins and proteoglycans carrying GAG chains[29]. Analogous to the differential expression of laminin isoforms, the collagen 'backbone' is associated with patchy expression of other ECM components, such as fibronectin and vitronectin, and their distribution likely creates preferred paths for interstitial migration and specific sites of tissue retention. For example, vitronectin is specifically upregulated in germinal centers (GCs) of the inflamed lymph node[59] and may facilitate the retention of $\alpha_V\beta_3^+$ follicular helper T ($T_{FH}$) cells in GCs[60]. In the lung, $\alpha_1\beta_1$ (also known as VLA1) and $\alpha_E\beta_7$ (also known as CD103) integrins play distinct roles in surveillance and epithelial positioning necessary for the retention of $T_{RM}$ cells[61,62].

## Physical and mechanical properties of ECM.

The ECM 3D ultrastructure provides a physical scaffold that facilitates or limits access to regions of the inflamed tissue and serves as a dynamic platform for the presentation of chemotactic cues (as discussed above) and growth factors[29]. Cytokines, such as tumour necrosis factor α (TNFα), interferon-γ (IFNγ) and transforming growth factor β (TGFβ), and proteases released in inflamed tissues modulate ECM density, composition and 'stiffness'. Thus, migrating leukocytes are faced with a highly variable inflammatory landscape (Fig 2). The technical advances afforded by microfabrication of 3D collagen matrices have facilitated the testing of migrational potential of T cells and have shed light on some of the decisions made by T cells when faced with directional choices[13,18]. However, our practical knowledge of ECM guidance *in vivo* is hindered by the inability to visualize the complexity of matrix components in situ in real-time in relation to the migrating T cell. Even basic questions of whether T cells are interfacing directly with the matrix, move over cells attached to the matrix or move between cells are unresolved. Thus, we remain quite ignorant of both the micro- and macro-topography over which T cells migrate in vivo.

Intravital imaging within tissues has revealed that T cells often follow the fibrillar collagen structures illuminated by second harmonic generation (SHG) [17,63], suggesting T cells can use the ECM ultrastructure to guide movement through the tissue. Indeed, in the context of toxoplasma infection, inflammation results in the de-novo generation of fibrillar matrix structures in the brain parenchyma that appear to facilitate the positioning of effector $CD8^+$ T cells at toxoplasma-infection foci[63]. Depending on the density of the fibre network,

and hence the degree of cellular confinement, T cells may migrate between fibres in an adhesion-independent fashion or along fibres requiring adhesion[8,64]. Indeed, inflammation-induced loosening of the collagen fibres in the dermis necessitated the use of matrix-binding integrins $\alpha_V\beta_1$ and $\alpha_V\beta_3$ by effector T helper 1 (Th1) cells for interstitial migration[17]. While changes to the ECM can facilitate movement within inflamed tissues, matrix changes surrounding solid tumours can have the opposite effect and serve to limit access to the tumour[65]. Human lung tumours are a striking example, where tumour islets are surrounded by a dense network of fibronectin fibres that constrain migrating T cells to a futile 'running track' around the tumour preventing T cell movement into the tumour itself[66].

Inflammation can also change the stiffness or elasticity of the tissue matrix impacting the mechanical forces applied to T cells as they migrate and the receptors used to sense such changes, in a process referred to as mechanosensing[67]. In general, cells tend to migrate toward stiffer environments due to increased traction forces moving from soft to stiff substrates[68]. The stiffness preferences for migrating leukocytes in 3D in vivo is unclear, in part due to the lack of tools to visualize individual matrix fibre rigidity. Studies using in vitro collagen matrices first showed that T cells can temporarily deform fibres coincident with their migration[13] and a similar reversible deformation of fibronectin fibres by migrating T cells was recently observed in vivo[69]. Interestingly, the interaction between cell and matrix can be reciprocal[12] with migrating cells inducing local matrix stiffening, opening up the possibility for guidance along self-fashioned stiffness gradients. Moreover, adhesion-based migration can leverage the elasticity of the matrix to enhance migration speed, the recoil from stretched fibres propelling rapid migration in a mode termed 'sling-shot migration'[70]. Whether these alternative forms of mechanical migration are employed by T cells as they move through inflamed sites remains to be tested.

### Antigen or chemokine-mediated arrest.

T cell migration in the inflamed tissue is shaped by the balance between positive migratory cues and negative arrest signals[71,72]; classically thought of as the balance between the strength of T cell receptor (TCR) ('stop') and chemokine ('go') signals. This competition was elegantly demonstrated in vitro where TCR-induced migratory arrest could be overridden by some, but not all, chemokine signals[73]. T cell arrest is dependent on LFA1–ICAM-1 mediated adhesion and TCR signalling that results in rapid actin cytoskeletal changes at the leading edge, reorientation of the microtubule organizing centre (MTOC), and the formation of a stable immunological synapse at the interface between the T cell and the APC[74]. At the molecular level, it remains unclear how stop and go signals are prioritized because both TCRs and chemokine receptors often share common downstream signalling intermediates. Differential sensitivity to negative regulators could be discriminatory, indeed the small GTPase RhoH appears to differentially regulate chemotaxis and TCR signalling, decreasing LFA1 adhesiveness for chemokine-mediated migration but enhancing prolonged contact with APCs[75]. Similarly, upregulation of negative modulators of T cell signalling such as cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD-1) can counteract TCR-mediated arrest and enhance T cell motility[76 77]. In contrast to the assignment of a 'go' function for chemokines, it is becoming clear that high concentrations of chemokines found near the source can restrict motility of cells by virtue of chemokine

receptor desensitization[71,78]. Such chemokine-mediated arrest appears to be critical for promoting T cell retention at infection foci. Effector $CD8^+$ T cells lacking CXC-chemokine receptor 3 (CXCR3) robustly migrated within the vaccinia virus-infected dermis but failed to stop at the CXCL9-enriched viral foci and showed reduced anti-viral function[79].

At inflamed sites, cognate antigen–MHC complexes on the surface of APCs represent a powerful stop signal. Indeed, intravital multiphoton imaging studies often use changes in the arrest coefficient of T cells as a surrogate for T cell–APC interactions[80,81]. In an in vivo delayed type hypersensitivity (DTH) model, where presentation of antigen could be synchronized, antigen-specific and dose-dependent effector T cell arrest was observed within 1 minute of cognate peptide administration[82]. High affinity antigen induces T cell deceleration via a process dependent on $Ca^{2+}$ signals and actin-related protein 2/3 (Arp2/3) activity[83]. In the absence of high affinity peptide–TCR interactions, T cells appear to adopt a migration mode that is more exploratory with a decrease in speed and frequent directional changes that may enable the integration of signals from multiple APCs or the search for alternative APCs displaying higher affinity ligands.

### Lipids mediators and other guidance cues.

The control of T cell migration by bioactive lipids is best known in the context of lymph node (and tissue) exit via lymphatics, where a sphingosine-1-phospate gradient from the lymphatics is sensed by the GPCR sphingosine-1-phospate receptor (S1PR1) expressed on activated T cells[84]. Inflammation-induced leukotrienes, in particular leukotriene B4 ($LTB_4$), are potent chemoattractants for leukocytes[85]. In parallel, the high affinity $LTB_4$ receptor, BLT1, is upregulated during T cell differentiation[86]. These lipid chemoattractants can be produced within minutes and are highly diffusible, thus their availability and tissue coverage may be far more ubiquitous than the targeted production of chemokines in the milieu. A number of new players in immune guidance and positioning are being discovered through the study of lymph node GC dynamics where the positioning and retention of $T_{FH}$ cell and B cells within the GC is critical for successful development of high-affinity antibody secreting cells[87]. GC organization is guided in part by oxysterol ligands at the follicle border and expression of the GPCR EBI2 by $T_{FH}$ cells and B cells[88]. GC B cell expression of a cell-bound guidance cue, plexin B2, appears to recruit $T_{FH}$ cells expressing the guidance receptor semaphorin 4C into the GC[89]. In addition to chemoattractants, several chemorepulsive mechanisms have been identified in regulating $T_{FH}$ cell GC dwell time, including $T_{FH}$ cell upregulation of S1PR2[90] and GC B cell expression of Ephrin B1[91]. Recently, B cell GC confinement was also shown to be shaped by the localized metabolism of a new glutathione ligand for the $G\alpha_{13}$-coupled receptor P2RY8 on B cells that inhibits cell migration[92,93]. The role that these new guidance cues play in non-lymphoid tissues is unclear, but they may influence the way in which T cells interact with antigen-bearing APCs or how T cells are retained in specific micro-anatomical niches. Uncovering the importance of these alternative guidance cues for T cell retention at sites of infection and chronic inflammation will provide a wealth of new therapeutic targets.

## Optimized T cell sensing

Optimum T cell guidance will depend on where and how the T cells mediate their effector functions in the inflamed tissue. $CD8^+$ T cell-mediated cytotoxic function requires direct cell–cell contact, which necessitates precise localization to infected cells. In contrast, $CD4^+$ T cells need to localize sufficiently close to the infection foci to be within the effective range of their secreted cytokines. For $T_H1$ cell-secreted IFN**γ**, its ability to induce inducible nitric oxide synthase (iNOS) expression in macrophages was estimated to extend some 80 μm from the site of antigen presentation[94]. The effective range of other key effector molecules, such as Th2 cell-secreted IL-4 and Th17 cell-secreted IL-17, remains to be determined. Thus, distinct $CD4^+$ effector T cell subsets may require individualized strategies to position themselves to best exert their effector function. While extrinsic guidance cues direct T cell migration, it is becoming clear that the motility of distinct T cell subsets is intrinsically tuned to optimize their ability to perform specific functions.

### Intrinsic programming of T cell migration.

Early studies described fundamental differences in the way in which $CD4^+$ and $CD8^+$ T cells scan the lymph node for initial activation signals[95] and how they migrate in the inflamed skin[96]. During activation and differentiation in the lymph node, subset-specific induction of chemokine receptors tailor responsiveness to chemokines expressed in particular effector niches within and between tissues[97]. $T_H1$ cells and $CD8^+$ T cells upregulate CXCR3 and this facilitates their repositioning to lymph node areas in which effector programming and lineage commitment processes are reinforced[98–100]. Upregulation of CXCR5 by $T_{FH}$ cell precursors similarly facilitates their positioning at the T–B border to receive further signals from B cells to ‘lock in’ the $T_{FH}$ cell programme[101]. For $T_H1$, $T_H2$ and $T_H17$ cell subsets, expression of distinct chemokine receptors also provide homing biases to respective tissue inflammatory milieus[102]. These homing biases are imprinted during $T_H$ cell differentiation through specific STAT and linage-specific transcription factor driven epigenetic regulation[103].

Effector T cells must balance the scope and intensity of exploration at inflamed sites to enable sufficient coverage of a tissue to detect rare targets, and sufficient time spent within each search region to promote productive cell–cell interactions[72]. Modified random walks, such as the Levy walk[14], observed for effector T cells in a variety of tissues are predicted to facilitate this balance; combining intermittent thorough search of a small region (confined) with extensive search of a broad area (periods of straight relocation). Although not well understood at the molecular level, T cell meandering appears to be intrinsically regulated in part by expression of myosin 1G (MYO1G) motors that regulate T cell intrinsic speed and the propensity to turn[104]. These unconventional myosin motors link actin filaments with the cell membrane and may act as molecular force sensors[105]. Loss of MYO1G resulted in T cell ballistic motility (faster and straighter) and the covering of more ground, but they were less able to detect rare targets[104]. The Rho-associated protein kinase ROCK also shapes the T cell meandering pattern, by regulating track straightness[106]. These findings highlight the functional significance of T cell-intrinsic tuning of the random walk.

More recently, T cell functional programing was shown to also extend to induced expression of differential motility machinery. $CD4^+$ T cell populations (such as $T_H1$, $T_H2$, $T_H17$, and $T_{reg}$ cell subsets) express subset-specific combinations of motility-associated genes[107]. Functionally, these differences change the requirement for environmental cues and correlate with different 'search' patterns within the inflamed tissue. STAT6-dependent upregulation of $\alpha_V\beta_3$ expression by $T_H2$ cells enables them to migrate through the inflamed dermis independent of GPCR signals. Conversely, $T_H1$ cell differentiation led to lower levels of $\alpha_V\beta_3$ expression necessitating chemokine/GPCR signalling for interstitial migration[107]. Subset specific differences in navigation within the inflamed target tissue may reflect differences in the way in which theses subsets deliver effector function. Indeed, in an independent study, tissue-enhanced $\alpha_V\beta3$ expression was linked to efficacy of $T_H17$ (but not $T_H1$) cell migration and pathogenicity in the CNS[108]. Although less well understood for effector $CD8^+$ T cell subsets, central memory $CD8^+$ T cells ($T_{CM}$), but not effector memory $CD8^+$ T cells ($T_{EM}$), express glycosyltransferase enzymes that generate functional ligands for E- and P-selectin binding that may promote rapid $T_{CM}$-specific entry into inflamed tissues[109], although this may not be a general rule[110]. These examples suggest that effector T cells acquire distinct motility set-points during initial activation in the draining lymph node that shape the way in which they subsequently respond to environmental factors within the infected target tissue.

### Sensitization and desensitization to extrinsic signals.

Our knowledge of how chemokines are 'seen' by T cells has lagged behind our ability to observe their migration *in vivo* due to the inability to visualize the gradients or measure the dynamics of receptor expression in real time, in situ. Standard genetic perturbation (knockdown, overexpression) is effective at identifying the receptors involved in a phenotype, but are less effective at extracting chemotactic mechanism. The steeply decaying gradients of GAG-associated chemokines only have an impact on directional migration over very short distances, in vivo just 50–75μm from the chemokine source itself[78,111]. Despite acting over small spatial distances, the directional sensing of this gradient has significant functional advantages for leukocyte positioning[112]. Increasing chemokine concentrations favour directional persistence and an increase in directional speed[78,113–115] allowing for persistent movement towards the chemokine source. At high concentration, chemokines can drive cellular arrest, due to the loss of gradient sensing or ligand-induced receptor internalization[71]. In this way, chemokines act as both attractants and restrainers of leukocyte motility making them potent positional cues within the inflammatory milieu.

Sensitivity to guidance cues is dynamically tuned at both ligand and receptor levels. This level of migration control is often overlooked in vivo due to limitations in visualizing and manipulating such changes in real-time. Yet, there are many ways in which chemoattractant gradients and the response to those gradients can be actively edited[71,116–118]. Such dynamic editing falls into three categories: removal or sequestration, post-translation modification (PTM) and/or receptor desensitization (Box 3). Local chemokine availability can be modulated by proteolytic degradation and by a growing number of atypical chemokine receptors (ACKRs) that act as scavenger or decoy receptors[119,120]. These receptors, expressed by stroma, endothelial cells and immune cells, bind and sequester

chemokines and thus play active roles in regulating chemokine bioavailability and shaping the chemokine gradient[116]. ACKR buffering of local chemokine concentrations may optimize the directional signal-to-noise ratio, ensuring robust cell migration in 'noisy' microenvironments[121]. This system has been co-opted by pathogens, with numerous pathogen-encoded chemokine decoy receptors that delay recruitment or disrupt positioning of critical immune effectors[119]. Adding to the complexity of chemokine sensing is an array of inflammation-induced PTMs including proteolytic truncation, nitration, citrullination and glycosylation[117]. These modifications dramatically alter the biological activity of the chemokine, promoting agonist or antagonist functions, modulating GAG-binding efficiency and changing receptor selectivity (Box 3). The impact of PTMs in vivo is as yet unclear, but therapeutic targeting may hold promise; small molecule blockade of CCL2 nitration in the tumour microenvironment augmented $CD8^+$ effector T cell tumour invasion[122]. The ability to sense chemoattractants is also regulated at the receptor level, with strategies that enhance or desensitize receptivity. Enhanced chemoattractant sensitivity can be achieved by re-positioning or clustering of chemokine receptors to the leading edge or filopodia of migrating cells[123], as seen in T cells with CXCR4 and active integrins being localized at the leading edge[124]. In contrast, both receptor internalization and desensitization of GPCR signalling[118,125] play an important role in modulating chemokine sensing[71], a process that is tightly regulated by GPCR kinases (GKRs) and by regulators of G protein signalling (RGS) proteins [114,125–127] (Box 3).

### Signal prioritization.

As outlined in the preceding sections of this review, T cells entering an inflamed site are met with a dizzying array of physical and chemical cues that are dynamically regulated by the inflammatory milieu (Fig 4). Coupled with tremendous redundancy in both receptor and ligand systems (for both chemoattractants and integrins)[128] our knowledge of how T cells prioritize spatiotemporal signals in complex environments in vivo remains understandably anecdotal. There is clearly a hierarchical response to competing chemokine gradients, physical guidance cues and arrest signals. At the molecular level, this can be established by competing downstream signaling[129,130] and the rate of receptor desensitization[131,132]. Differential receptor desensitization can impose ligand biases[133] that may also impose a spatial bias[134]. Live imaging of receptor internalization in vivo, revealed differential internalization of CXCR1 and CXCR2 by neutrophils in response to CXCL8 ligand that prevented overt neutrophil 'congregation' at the wound site[134]. A nascent understanding of crosstalk between receptors of different guidance cues is emerging with examples of chemokines regulating integrin ligand specificity[135] and integrin expression levels modulating the dependency on chemokine signalling[107]. How physical cues are prioritized is not well understood, save for the physical and mechanical restrictions previously discussed, but there was an interesting study that showed that local hydraulic pressure (introduced by inflammation-driven oedema) could override chemotactic cues for directional migration[136]. The above mechanisms of dynamic tuning of sensitivity to environmental cues should enable T cells to dynamically regulate speed and directionality 'on the fly' and to integrate and prioritize such heterogeneous signals.

## Amplifying guidance cues for T cell positioning

The ‘prize’ for successful integration of these overlapping guidance cues is the correct positioning of effector T cells for delivery of effector function and their timely dispersal for the resolution of inflammation. Key to success is the ability to optimize the detection of ‘targets’ (ligand-bearing APCs, infected or damaged cells) within a crowded cellular microenvironment. Lessons learnt from studies of patterns of foraging for food, suggest that the distribution of the targets within inflamed tissues will dictate successful strategies[137]. In ant models, the distribution of targets appears to dictate the necessity for ant-to-ant communication; patchy or clustered resources requiring cooperative signalling between foragers via pheromones that increases the ease and completeness of the search. While it has largely been assumed that antigen encounter is a relatively rare event at infection sites[80,138], the observed clustering of T cells and APCs at sites of inflammation may serve to amplify the target for ease of search (Fig. 4).

### Cellular collaboration.

For neutrophils, well-orchestrated spatial and temporal waves of attractants help guide precise positioning to sites of tissue damage. End-target attractants originating from damaged/dying cells or bacteria provide precise microanatomical coordinates, while secondary attractants produced by endothelial cells or other immune cells at the inflamed site promote recruitment and migration within the tissue[116]. Remarkably, the relay of these signals can be autonomously controlled, with the ‘hand off’ of signals between neutrophils occurring as they migrate by secretion of $LTB_4$ at the rear of one migrating cell promoting the migration of the neutrophil next in line[139,140]. This relay system substantially increases the recruitment range of cells and supports directionality over long distances[116]. For T cells, the relay of signals requires collaboration between immune cell types, shaped by resident or recruited innate cells entering the tissue hours to days before the arrival of the T cells themselves. Indeed, neutrophils can leave long-lasting membranous trails containing CXCL12 that inform $CD8^+$ T cell migration paths in the influenza-infected airway[15]. More recently, dying neutrophils appear to ‘hand off’ signals to infiltrating inflammatory monocytes that optimizes $CD8^+$ T cell activation for anti-viral activity[141].

Akin to end-target chemokines of neutrophils, it’s becoming increasingly clear that chemokine production by APCs, or co-clustering of antigen-bearing APC with chemokine-producing inflammatory monocytes or stroma, provides a powerful ‘end-target’ positioning cue for T cells at all functional stages. Production of CCL3 and CCL4 by antigen-specific $CD4^+$ T cell-DC clusters in the lymph node makes for a more attractive target for initial $CD8^+$ T cell activation, enhancing the $CCR5^+$ $CD8^+$ T cell-DC ‘hit rate’ and promoting $CD8^+$ T cell memory[113]. $T_H$ cell differentiation is optimized by positioning of activated T cells at peri- or inter-follicular regions of the lymph node, dependent on $CXCL9^+$ stroma and $CXCL10^+$ DCs for $T_H1$ cells[100] and on CXCL13 and $CXCL5^+$ DCs for $T_H2$ cells[142]. In infected or inflamed tissues, the CXCR3 ligands CXCL9, CXCL10 and CXCL11 are produced by DCs, macrophages and nonhematopoietic cells and also guide sub-anatomical positioning to targets[14,79,143,144]. This critical protective mechanism can be amplified by an IFN$\gamma$-dependent positive feedback loop from strategically positioned effector T cells[145] and

is often actively subverted by pathogens to evade detection. Moreover, at the memory stage, rapid CXCR3-dependent relocation of central memory $CD8^+$ T cells within the lymph node boosts early anti-viral recall responsiveness[146,147].

## Specialized tissue niches.

While the individual migratory paths of T cells are inherently random, recent findings suggest that encounters with antigen are far from arbitrary. Pre-positioned niches in the steady state optimize antigen encounter and 'pop up' activation niches in inflamed tissues provide hubs for signal integration. In the lymph node, strategic positioning of macrophages and DCs (in particular cDC2s) at the lymphatic sinus or subcapsular sinus floor facilitates the sampling of lymph-borne particulate materials coming from peripheral tissues and facilitates early activation of B cell and T cells, respectively[148–150]. Rapid redeployment of lymph node-resident DCs to these sites may also potentiate the activation niche[151]. More recently, naive $CD4^+$ T cells were shown to have a similar steady-state spatial preference at the periphery of the lymph node paracortex regulated by the expression of the GPCR Ebi2[152]. This 'anatomical platform' appears to optimize naïve $CD4^+$ T cell encounters with MHC class $II^+$APCs for early activation. In many non-lymphoid tissues, a similar steady-state strategic positioning of resident lymphoid cells, such as innate lymphoid cells (ILCs), appears to facilitate early immune responses to damage or infection. ILC niches have been found in multiple organs including the barrier tissues of the skin, lung and gut and are composed of colocalized tissue stroma, peripheral nerves, resident innate immune cell types and regulatory T cells[153]. Crosstalk between cell types in the ILC niche potentiates or regulates initial immune activation[154–156]. The perivascular adventitial cuff has also emerged as an interesting microanatomical site where tissue-derived signals (TLR ligands, antigen, cytokines) carried in the draining interstitial fluid meet resident DCs and ILCs poised to respond[157]. Disrupting the adventitial stromal cells in this niche impaired helminth-induced ILC2 expansion and Th2 cell accumulation and function[154]. Whether these regional hubs designed for early innate immune sensing also provide a platform for the subsequent re-activation of incoming effector T cells is unclear at present. Nonetheless, these hubs, enriched in APCs and chemokines, are scattered throughout the tissue and could represent important tissue "outposts" for optimized presentation of antigens to newly recruited effector T cells[157].

The widely observed clustering of effector T cells with APCs in inflamed peripheral tissues in human disease and mouse models suggests that spatial preferences for T cell activation also exist at inflamed and infected sites[80,158–160]. These activation clusters may simply mark the location of the pathogen itself, or represent induced hubs for peripheral re-activation. Indeed, the perivascular clustering of macrophages, DCs and effector $CD8^+$ T cells was shown to be essential for peripheral activation of T cells in the inflamed skin during contact hypersensitivity[161]. These perivascular clusters appear to represent de novo generated activation platforms, induced by IL-1α-triggered macrophage production of CXCL12 that initiates DC clustering. Similarly, local $CD8^+$ T cell expansion in the liver following acute viral infection was shown to occur in myeloid-cell aggregates induced by TNF[162]. The guidance cues required for effector T cells to accumulate within the perivascular clusters are unclear. T cells could use the clusters as sites of preferred tissue

entry or be recruited to the site following entry elsewhere. Given their perivascular location, such specialized niches may reduce the scope of tissue needed to be searched by T cells early on in the immune response and could serve as initial local sorting houses, optimizing the peripheral expansion of 'useful' antigen-specific effector T cells. The spatial relationship between such activation clusters and subsequent delivery of effector function at infection foci remains to be determined. The functional advantage to non-random spatial distributions within tissues is perhaps best demonstrated by the strategically positioned $T_{RM}$ cells. At some mucosal tissues, $T_{RM}$ cells are maintained within tight clusters[163,164], defined in part by chemokine-rich myeloid niches[164]. Due to their proximity to the mucosal surface, these memory hubs appear to serve as a local 'immediate response centres' for early detection of invading pathogens.

## Closing remarks

Remarkable progress has been made in recent years on the mechanics of T cell migration at the single cell level and on defining the complexity of modulating factors in the inflammatory milieu that may shape migratory paths of effector T cells in target tissues. These studies have provided insight into the physiologic and pathologic migration patterns of T cells and their role in health and disease. Recent examples of strategic pre-positioning and induced micro-anatomical niches for immune activation at inflamed sites, provide a new conceptual framework for understanding how migration and positioning of effector T cells is optimized, and should help shape a more 'surgical' approach for site-specific intervention strategies.

Advanced imaging has enabled the sub-cellular analysis of molecular pathways as T cells migrate and facilitated the identification of sub-anatomical niches within whole tissues. Yet, the details of the molecular presentation patterns of T cell migration signals have not been clearly determined in intact tissues. How are micro- or macro-chemokine gradients established and maintained at sites of tissue infection? Does specific integrin ligand expression have any role to play in the ability of T cells to home and stay at the specific site of the tissue injury? How do effector T cells rapidly integrate the diverse and often redundant local mechanical signals with chemoattractant signals for migration within inflamed tissues? How does T cell intrinsic programming impact on this decision? Layered on top of these questions regarding basic mechanisms of migration in situ, are spatial and temporal considerations. We remain ill-informed regarding points of T cell tissue entry relative to target distribution and thus are unclear as to the range of tissue explored by incoming effector T cells. Furthermore, while the need for T cell peripheral reactivation by APCs is clear, the position of these reactivation events relative to the location at which effector function needs to be delivered is poorly understood. Current immunotherapies for the treatment of various cancers have exposed some of our 'blind spots' when it comes to understanding how effector T cells access and position themselves within inflamed tissues. The clinical efficacy of cancer immunotherapy strongly correlates with the number of T cells that infiltrate the tumour microenvironment, but the monitoring of chimeric antigen receptor (CAR) T cells after adoptive cell transfer has shown that only ~1% of the total transferred T cells initially migrate into the tumour. Therefore, it appears we have some ways to go before we can rationally exploit T cell migration strategies for clinical benefit.

In conclusion, our conventional understanding of T cell migration based on individual modules (chemoattractants, integrin activation and biophysical confinement) is far too simplistic. While there are numerous new and creative therapeutic strategies based on targeting T cell migration, success thus far has been modest (Box 4). T cell migration in vivo faces complex challenges from both cell-intrinsic factors that can directly modulate migration signals and cell-extrinsic cues arising from the inflammatory microenvironment. Specificity and flexibility of T cell migration is essential at multiple stages: successful pathogen clearance, prompt resolution of inflammation and the establishment of locally positioned memory potential, all while minimizing collateral tissue damage. A better understanding of the combinatorial biochemical decision making that occurs in support of T cell migration and of the local guidance cues that recruit and retain effector T cells in the correct sub-anatomical location will open up new therapeutic possibilities for inflammatory diseases and enhance the effectiveness of existing clinical approaches.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Box 1.**

### Actomyosin-based 3D migration.

At the molecular level, T cell migration involves applying internally generated force to generate traction against the extracellular environment for forward movement. Cellular polarization and actin retrograde flow is regulated in part by chemokines via GPCR signalling that drives an F-actin-rich leading edge and an acto-myosin rich uropod[64]. While chemokines can lead to quantitative increases in actin flow, chemokines alone are insufficient to drive forward locomotion, leaving the cells to 'run in place'[52]. Tension with the extracellular environment is required for forward motion and can be achieved by active engagement of adhesion receptors such as integrins that act as a clutch to engage the actin motors (See Box 1 figure). Integrin binding to matrix ligands is coupled to the actin cytoskeleton via intracellular adaptor proteins such as kindlin and talin. Integrin engagement requires an activation event in which the integrin undergoes a conformational change from a closed, bent, form to an open, extended, form that has high binding activity[51]. Chemokine signalling serves to activate integrins by regulating the binding of talin to the integrin cytoplasmic tail and to actin itself. In reciprocal fashion, actin engagement creates tension or 'pulling' on the intregin β-chain to stabilize the active integrin conformational state[165]. For rapid T cell movement in 3D, T cells must balance force generation and adhesion to tune their response to the microenvironment. This appears to be achieved by a relative low integrin mediated force transmission by a seemingly dispersed network of integrins, possibly through highly dynamic micro-adhesive contacts that can provide sufficient friction to enable T cells to rapidly adapt to micro-anatomical terrains without losing momentum. More recently, non-integrin adhesion receptors or a non-receptor friction interface, created by confinement and surface texture or topography, has also been found to create sufficient shear forces to move the cell forwards[31].

**Box 2.**

## Physical constraints

The interfibrillar space of the extracellular matrix (ECM) influences the direction and mechanisms used for 3D migration and can vary dramatically in different tissues (for example, from loose connective tissue in the spleen, intestinal lamina propria and lymph nodes to extremely dense basement membrane tissue) with spaces ranging between 2 – 30 μm (See Box 2 figure). While it is known that cell speed is a linear function of pore size, in vivo measurement of physical size constraints for 3D migration T cells is limited. For movement into the tissues, the minimum endothelial pore size during T cell transmigration was estimated at 5.1 – 5.4 μm[38,166]. Below a critical capillary size of approximately 4.5 μm, T cells appear to get trapped or can rupture[19,167]. * The apparent Young's modulus $E_{app}$ represents a static estimation of the elastic behavior of the material. A human T cell elasticity, or 'softness', of ~85 Pa was shown to be considerably lower than that of monocytes (~520 Pa), dendritic cells (~440 Pa) and macrophages (~900 Pa)[19].

**Box 3.**

## Dynamic regulation of chemokine sensing

**Chemokine removal or sequestration.**

The family of atypical chemokine receptors (ACKRs) include ACKR1 (also known as DARC), ACKR2 (also known as D6), ACKR3 (also known as CXCR7) and ACKR4 (also known as CCX-CKR)[119]. These receptors, which are often expressed by endothelium and immune cells, bind chemokines but do not couple to G proteins for GPCR signalling for cell migration. In part, ligand-dependent endocytosis targets the chemokine for degradation[116]. The scavenger receptor CXCR7 acts to buffer local chemokine concentrations by matching the attractant concentration to the receptor $K_d$ to optimize the directional signal-to-noise ratio[121]. Models of ACKR genetic ablation result in overt T cell accumulation in peripheral tissues[120].

**Post-translational modifications (PTMs).**

Inflammation-induced PTMs of chemoattractants include proteolytic truncation, nitration, citrullination and glycosylation. **Truncation** at the $NH_2$ or COOH-terminus increases, decreases potency or changes receptor specificity. Examples include: CXCL8 and CXCL5 truncation by matrix metalloproteinases (MMPs), which increases potency of neutrophil chemotaxis[168]; CXCL10 and CXCL11 truncation by CD26 or CD13, which inactivates chemokine-mediated T cell chemotaxis and recruitment[169–171], antagonizing CXCR3 and disrupting GAG binding; CCL5 truncation, which alters receptor specificity, abolishing CCR1 and CCR3 binding but maintaining CCR5 binding[172]. **Nitration** alters affinity for GPCRs and GAGs: CCL2 nitration reduces affinity for both the chemokine receptor and GAGs[173] and blocks $CD8^+$ T cell recruitment to tumours[122]. **Citrullination** alters the charge and interactions with GPCRs, GAGs and lipid: CXCL10 and CXCL11 citrullination reduces potency of CXCR3 signalling and reduces its capacity to bind GAGs, attenuating T cell migration[174]. Receptor modification also alter function: polysialylation of CCR7 regulates DC migration by enhancing CCL21 activity[175] and TLR-induced upregulation of dihydroceramide leads to ceramide-dependent inversion of the topography of CCR5 in the ER of macrophages, preventing their migration towards CCL5[176].

**Receptor desensitization.**

Ligand-induced receptor internalization is regulated by: clathrin/β-arrestin mediated internalization for degradation or recycling[118]; GPCR phosphorylation by GPCR kinases (GRKs) which can couple the GPCR to arrestins, resulting in steric inhibition of receptor interactions with G proteins[114,125,126]; and by regulators of G protein signaling (RGS) proteins that accelerate the activity of G protein GTPases[127].

## Box 4.

### Therapeutics targeting effector T cell migration

**Blocking tissue entry to mitigate inflammation.**

Highly activated pro-inflammatory effector T cell migration is involved in a wide range of pathologic conditions, including vasculitis, atherosclerosis, stroke, rheumatoid arthritis, lupus, multiple sclerosis and Crohn's disease[177–179]. Thus, T cell recruitment receptors (such as integrins and chemokines) have been key targets for novel therapies aimed at inhibiting the dysregulated immune response in autoimmune and allergic conditions.

#### Integrins.

Four integrins expressed exclusively in leukocytes ($\alpha_L\beta_2$, $\alpha_4\beta_1$, $\alpha_4\beta_7$, and $\alpha_E\beta_7$) are involved in the recruitment and retention of effector T cells at many tissue sites during inflammation. ***Natalizumab:*** a humanized antibody to $\alpha_4$ integrins, was shown to alleviate multiple sclerosis, but was withdrawn after seeing a few fatal cases of progressive multi-focal leukoencephalopathy (PML) likely due to an iatrogenic immune-defect by blocking $\alpha_4$ integrins that would be permissive for reactivation of a latent human polyoma virus 2 (also known as JC virus) infection[180,181]. ***Vedolizumab***: selectively blocks $\alpha_4\beta_7$ integrin and showed a significant clinical benefit in the treatment of inflammatory bowel diseases. ***Efalizumab:*** targets the integrin $\alpha_L$ subunit and was originally developed for psoriasis, then was withdrawn in 2009 because of an association with PML. ***Etrolizumab:*** a humanized monoclonal antibody developed to selectively block the $b_7$ integrins ($a_4b_7$, and $a_Eb_7$). Etrolizumab met its primary endpoint of inducing remission versus placebo for patients with ulcerative colitis in a few clinical studies. A phase III study in Crohn's disease is currently ongoing.

#### Chemokines.

The chemokine family has attracted great interest for treatment of inflammatory diseases, as it comprises major players in the determination of the tissue-specificity and selectivity during T cell recruitment. However, although G-protein-coupled receptors (GPCRs) represent one of the most successful targets for the drug discovery, intense efforts for several chemokine receptor inhibitors have failed to deliver fruitful drug candidates. Until now, chemokine receptor antagonists have only been clinically approved as antiviral agents (for example, CCR5 and CXCR4 antagonists for HIV). AMG487 is a potent CXCR3 antagonist developed to treat psoriasis. However, a Phase II efficacy and safety study with AMG487 later has been terminated due to the lack of efficacy.

**Recruitment to tumours for T cell immunotherapy.**

Many types of tumors can actively prevent T cell infiltration by modifying the expression of adhesion molecules in vascular endothelial cells[182] or inducing posttranslational modification of local chemokine signals[122]. Several recent studies proposed novel molecular checkpoints that could be harnessed to improve homing of T cells to their target sites, thus, to increase the efficacy of T cell immunotherapy, while reducing the risks associated with non-specific cytotoxicity. For example, an oncolytic

vaccinia virus engineered to produce chemokine CXCL11 increased the chemokine expression level within tumor sites, and successfully recruited T cells and augmented anti-tumor efficacy in a mouse preclinical study[183]. A CXCL10-loaded folate-modified chitosan nanoparticle showed anti-tumor T cell responses and reduced the growth of hepatocellular carcinoma in mice[184]. Targeted delivery of tumor necrosis factor superfamily member, LIGHT, eradicated established tumors in mice by activating lymphotoxin-β receptor and facilitating local production of CCL21 and CXCL13 that led CTLs into the tumor site (Tang Cancer Cell 2016). Epigenetic reprograming of the production of chemokines CXCL9 and CXCL10 has been attributed to poor T cell infiltration to the tumor microenvironment[185]. GSK126, a selective inhibitor of EZH2 methyltransferase activity, and DZNep, an all *S*-adenosyl-methionine-dependent enzymes inhibitor increased production of T cell trafficking chemokines and augmented the therapeutic effects of PD-L1 blockade. In a pancreatic cancer mouse model, AMD3100, a CXCR4 small-molecule inhibitor led to improved T cell migration to the tumor site and enhanced the anti-tumor effects of an anti-PD-L1[186]. Similarly, LY2510924, a potent and selective peptide antagonist of CXCR4, resulted in selective reduction of intratumor Treg cells and showed a synergistic effect with PD-1 inhibitors in a syngeneic squamous cell carcinoma model[187]. Based on these pre-clinical data, an open-label phase Ia study testing the safety of combination therapy with LY2510924 and the anti-PD-L1 antibody durvalumab in patients with advanced refractory solid tumors was recently completed[188]. Although the intratumoral delivery of a chemokine-encoding system has been proposed to enhance CTL homing to the tumor site in several pre-clinical studies, it is important note that chemokines directly contribute to tumor growth, metastasis, and angiogenesis[189–191]. Thus, it is critical to selectively and precisely control chemokine signals only in the tumor-targeting T cells to avoid potential adverse outcomes.

**Boosting tissue resident T cells to promote vaccine efficacy.**

Precise control of T cell migration and retention is a key to the development of effective vaccines as well as treatment of immune-mediated diseases. As we discussed above, direct applications of exogenous chemokine signals have been proposed to recruit a specific subtype of effector T cells into target tissues[192]. The functional importance of tissue resident memory T ($T_{RM}$) cells has driven a novel immunization approach designed to ‘prime and pull’ T cells into tissues[193]. This technique combines two steps: conventional parenteral vaccination (prime) and topical administration of chemokines or adjuvants to recruit activated T cells to target tissues (pull)[194,195]. Recently, additional ‘prime and target’ approach utilizing an organ specific antigen-expressing system provided similar proof-of-principle results[196], suggesting that protective $T_{RM}$ cells can be generated through this promising tissue-specific T cell recruitment strategy.

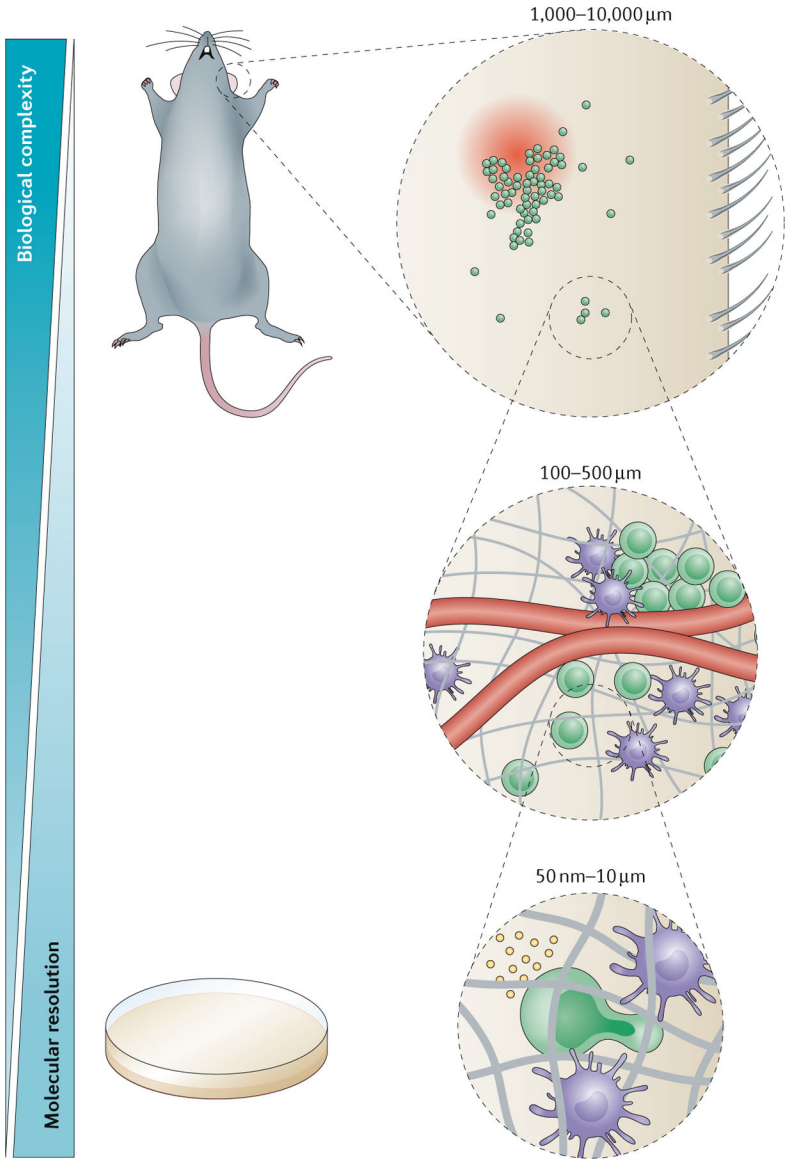


**Figure 1. The study of T cell migration: a trade-off between molecular resolution and biological complexity**

The tool box for in situ analysis of T cell migration is growing rapidly thanks to innovations in resolution through tissue-clearing techniques and super-resolution imaging modalities. Increasing molecular resolution often results in loss of biological complexity. At one end of the spectrum, the use of photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) facilitates single molecule analysis in whole cells, enabling the determination of conformational changes in single integrin heterodimers in migrating cells. Use of total internal reflection fluorescence microscopy (TIRF) microscopy has enabled the study of the dynamics of force transmission close (within ~100nm) to the plasma membrane of migrating cells. Real-time 3D analysis of T cell surface topography is possible with light-sheet microscopy. These tools have provided critical insight into molecular mechanism, but cannot be utilized in complex 3D tissues of the living animal. At the other end of the spectrum, advances in 3D histology using tissue

clearing techniques and multiplex confocal microscopy has provided an unprecedented look at antigen dispersal and immune cell position in 3D, but the dynamics of T cell migration are lost. In between, the use of intravital multiphoton microscopy provides the ability to visualize T cell migration in real-time in the context of tissue complexity (albeit for relatively short periods of time, hours). Development of photoactivation tools and force sensors to assess molecular details and manipulate signals in individual migrating cells in real time by multiphoton will help to bridge these gaps in scale.

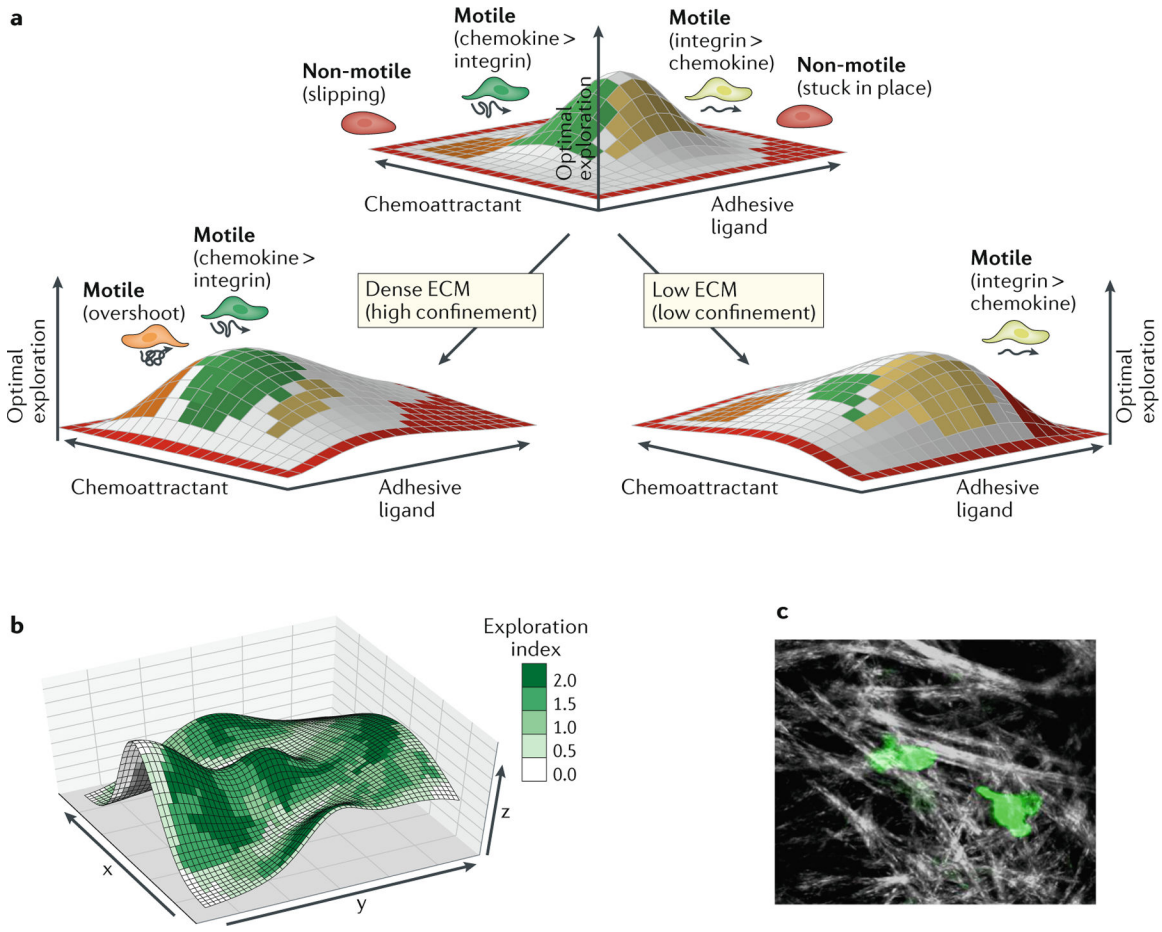


**Figure 2. Mode of migration is shaped by input from multiple signals**

(**A**) A phase space diagram illustrating the cooperative behaviour of chemoattractants, adhesive ligands and the degree of confinement or tissue topography in promoting or inhibiting cell motility and tissue exploration. The upper diagram is based on a gaussian function with two variables (chemoattractant versus adhesive ligand). The presence of one parameter without the other is unable to support motility. High levels of adhesion or chemoattractant result in T cell arrest by being stuck in place or 'spinning their wheels', respectively. The shaded regions indicate migration modes occupied by T cells that favour chemokine or adhesion dependency. The lower diagrams introduce a third variable, confinement, and illustrate how the peak (optimal exploration) moves towards chemokine-based efficiency under high confinement associated with dense ECM (left) and towards adhesion-requirements under low confinement (right). **(B)** 4D landscape model of 3 variables (*X, Y, Z*) with a fourth functional dimension of exploration efficiency. In vivo, gaussian functions and simple relations between each variable are unlikely, rather the tuning of T cell exploration in the tissue will be determined by local microdomains resulting in a highly variable navigable landscape. Here *x, y, z* are hypothetical variables as there is insufficient data to map integrated responses to known guidance cues, but each variable would represent an individual chemokine, integrin ligand or specific mechanical parameter. **(C)** Intravital multiphoton microscope image of migrating Th1 cells (green) in a dermal collagen network (white, second harmonic generation (SHG) to illustrate the variability of

just one visible parameter, physical confinement, in vivo. Although in reality, these cells are integrating signals from multiple ‘hidden’ factors such as chemokines and integrin ligands.

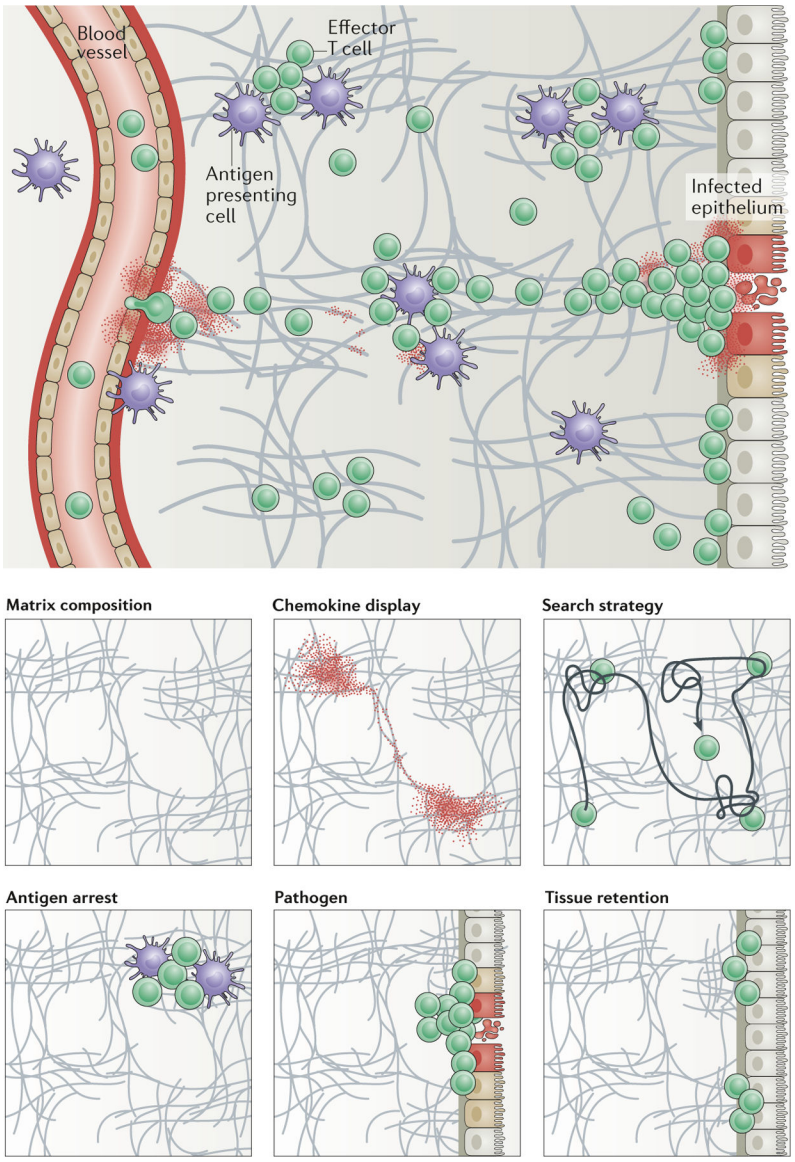


**Figure 3. Regulation of effector T cell migration within inflamed tissues**

Successful tissue immunity requires efficient T cell migration within the inflamed tissue. The migratory path is shaped by multiple guidance cues spatiotemporal displayed in distinct microenvironments. At the tissue level, Effector T cells (green) must undergo extravasation or transendothelial migration from the blood into tissue, cross the basement membrane, ‘search’ the inflamed tissue for antigen-bearing target cells (antigen presenting cell, purple) for peripheral reactivation and exert effector function at foci of infection. This migratory path is influenced (promoted and inhibited) by: the density and composition of the matrix; the multivariate and dynamic display of chemoattractants; effector T cell intrinsic motility programming that pre-sets receptivity to guidance cues and optimizes the ability to ‘search’ for tissue targets; the density and distribution of antigen-bearing targets; pathogen-specific infection niches and the ability to retain T cells for rapid recall.

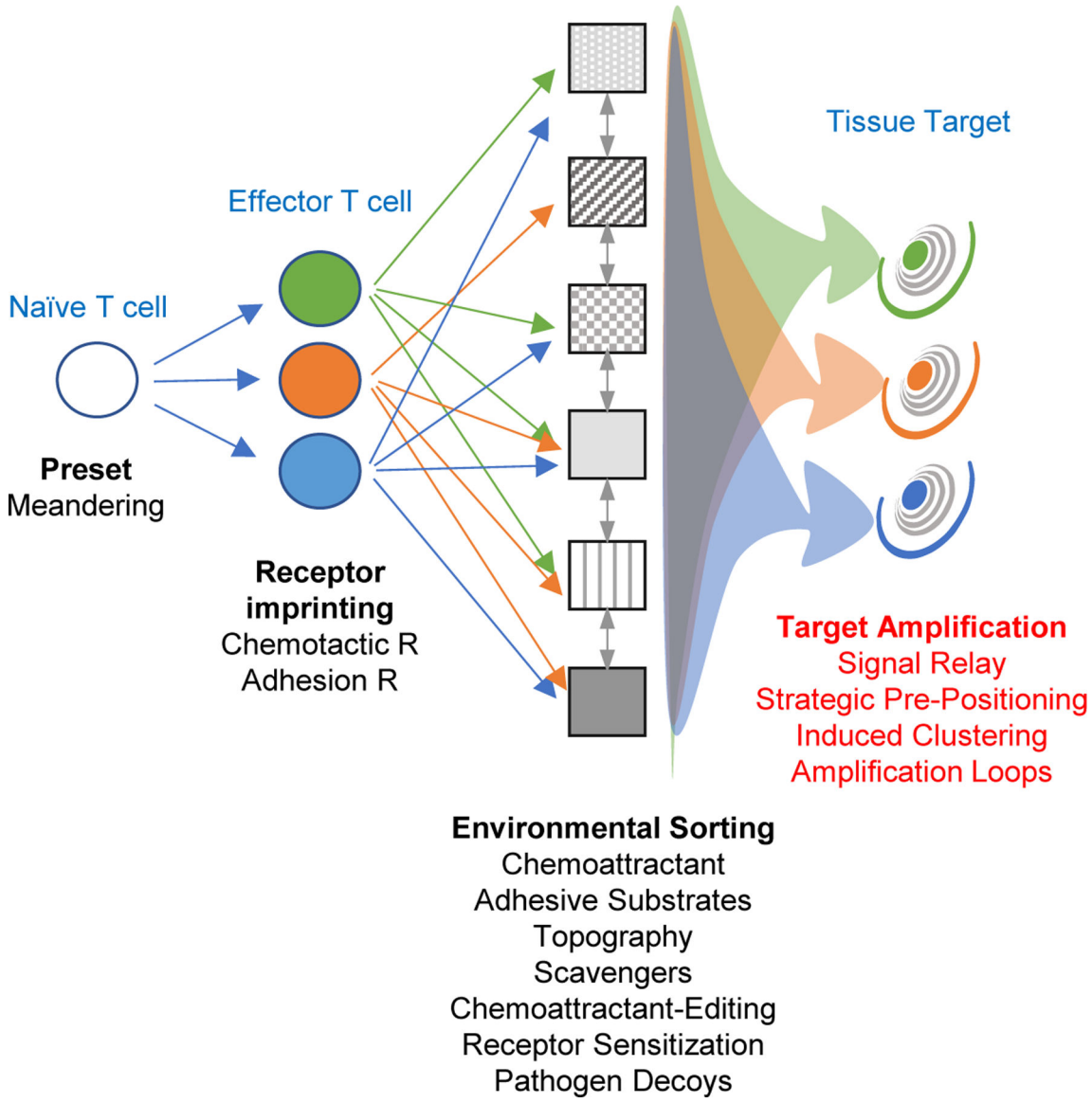


**Figure 4. Spatiotemporal optimization of effector T cell positioning**

A summary of the control points that build an efficient effector T cell response. The complexity of external cues and T cell receptivity to these cues is increased by the inflammatory milieu and by T cell differentiation, but can be honed at the tissue site by spatiotemporal mechanisms that appear to amplify the target to optimize effector T cell-specific positioning. Receptivity is enhanced as T cells differentiate from naive to effector T cells (for example, Th1, Th2, Th17 cells) in the lymph node where intrinsic programming drives expression of receptor and signaling machinery that promotes biased receptivity to guidance cues at the inflamed tissue. Once the effector T cells enter the inflamed tissue, a sharp increase in complexity of external cues at the tissue site is driven by a host of competing guidance cues (indicated by the shaded gray boxes) that promote or restrict the ability of effector T cells to ‘search’ the inflamed tissue. This complexity is functionally simplified by non-random spatial clustering of antigen and guidance cues

within the inflamed/infected tissue, that appears to amplify the target in an effector T cell-specific way, by reducing the scope of the tissue search and promoting local retention.