Regulation of global CD8+ T-cell positioning by the actomyosin cytoskeleton

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Summary

CD8+ T cells have evolved as one of the most motile mammalian cell types, designed to continuously scan peptide–major histocompatibility complexes class I on the surfaces of other cells. Chemoattractants and adhesion molecules direct CD8+ T-cell homing to and migration within secondary lymphoid organs, where these cells colocalize with antigen-presenting dendritic cells in confined tissue volumes. CD8+ T-cell activation induces a switch to infiltration of non-lymphoid tissue (NLT), which differ in their topology and biophysical properties from lymphoid tissue. Here, we provide a short overview on regulation of organism-wide trafficking patterns during naive T-cell recirculation and their switch to non-lymphoid tissue homing during activation. The migratory lifestyle of CD8+ T cells is regulated by their actomyosin cytoskeleton, which translates chemical signals from surface receptors into mechanical work. We explore how properties of the actomyosin cytoskeleton and its regulators affect CD8+ T cell function in lymphoid and non-lymphoid tissue, combining recent findings in the field of cell migration and actin network regulation with tissue anatomy. Finally, we hypothesize that under certain conditions, intrinsic regulation of actomyosin dynamics may render NLT CD8+ T-cell populations less dependent on input from extrinsic signals during tissue scanning.

KEYWORDS
lymphoid and non-lymphoid tissues, extracellular matrix barriers, naive and effector/memory CD8+ T-cell trafficking, regulation of actomyosin cytoskeleton

1 | PART 1. IN VIVO CD8+ T CELL ACTIVATION AND EXPANSION INVOLVES ORGANISM-WIDE TRAFFICKING PATTERNS

The adaptive immune system faces the formidable challenge to respond to a highly diverse variety of pathogenic organisms that have breached epithelial barriers or pose a potential danger. Signature molecular patterns derived from pathogens bind to cognate pathogen-associated molecular pattern (PAMP) receptors expressed by innate immune cells to inform them on the presence and nature of the threat. The characteristics of the PAMP signaling and other cues induce adaptive immune reactions, which take into account pathogen size, and therefore its susceptibility to phagocytosis, the intra-vs extracellular location as well as the anatomical site of pathogen entry (eg, gut vs skin). Based on this information, the ensuing adaptive immune responses can be classified into four types.[1] *Type II* immune responses are triggered by extracellular, multicellular organisms such as helminths, which are too large to be phagocytosed. *Type III* immune responses are triggered by extracellular bacteria and fungi, once they have breached epithelial barriers such as the epidermal layer of skin. *Type IV* immune responses are elicited against

extracellular pathogens before these have breached body barriers, such as against gut microbiota. Here, we focus on *Type I* immune responses, which are specifically directed against intracellular pathogens. These include some bacteria and parasites, such as Listeria monocytogenes and plasmodium, respectively, which have evolved to thrive inside host cells. Yet, the most common trigger for *Type I* immune responses are viral infections, which may affect any nucleated cell anywhere in the body including hematopoietic and stromal cell types. In recent years, it has emerged that cancerogeneous cells are also eliminated in this kind of immune response, which is exploited in checkpoint inhibitor therapies. *Type I* immunity involves IFNγ-secreting Th1 CD4$^+$ T cells, inflammatory "M1" macrophages, IgG-producing B cells, NK cells, and cytotoxic CD8$^+$ T cells. CD8$^+$ T cells recognize cognate class I peptide–major histocompatibility complexes (pMHC) presented on the surface of antigen-presenting cells (APC) during priming and on infected or cancerogeneous cells in target organs. This is because class I pMHC reflect the intracellular proteome including pathogen-derived non-self peptides or peptides from neoantigen proteins produced by mutations of the genome. Once engaged by the recognition of cognate class I pMHC, effector CD8$^+$ T cells (T$_{EFF}$) induce apoptosis of target cells. Thus, *Type I* immune responses uniquely involve the directed killing of host cells to reduce or eliminate viral reservoirs. To accomplish this feat, naive CD8$^+$ T cells have to identify rare APC, usually dendritic cells (DCs), to become activated and generate large numbers of cytotoxic CD8$^+$ T$_{EFF}$ during clonal expansion. Once released into the circulation, CD8$^+$ T$_{EFF}$ perform a "search and destroy" mission directed against their own host cells. After contraction, memory CD8$^+$ T cells continuously patrol lymphoid and non-lymphoid tissues (NLT) for rapid recall responses.

The process of target cell elimination has been extensively studied in simplified in vitro systems. In sum, effector CD8$^+$ T cells directly bind to the target cell expressing cognate pMHC to directionally deliver the content of their cytotoxic granules including perforin and granzyme B. Pores in the target cell membrane created by perforin and other molecules allow granzyme-triggered caspase activation, which in turn sparks a cell-autonomous apoptotic program.[2] The close juxtaposition of target cell and CD8$^+$ T cell ensures high-precision, selective killing through apoptosis and subsequent elimination of cellular and viral particles by macrophages. This process also avoids excessive inflammation associated with pyroptosis or necrosis. Recent work has identified actomyosin cytoskeleton-mediated force as an important mechanism to potentiate cytotoxic T-cell activity. Thus, once a target cell is identified, retrograde F-actin flow at the immunological synapse (IS) enhances the pore-forming activity of perforin.[3] Furthermore, cortical actin network reorganization at the IS facilitates centrosome repositioning and granule polarization required for targeted delivery of the cytotoxic cargo to the target cell.[4] Taken together, the "destroy" part mediated by CD8$^+$ T$_{EFF}$ cells is well characterized on a cellular and molecular level, including biophysical force generation.[5-7]

What about the "search" part? Numerous studies have uncovered the remarkable adaptability of CD8$^+$ T cells to disseminate into non-lymphoid organs during an immune response, leading immunologists to take their tissue infiltration capacity as a given. Yet, it is now recognized that CD8$^+$ T cells may be excluded from tissues. Truncated presence of CD8$^+$ T cells and other leukocytes in solid tumors strongly correlates with a poor prognosis for successful checkpoint inhibitor therapy.[8-10] These observations have rekindled interest in how cytotoxic T cells find their target cell inside complex microenvironments in vivo. pMHC molecules are transmembrane proteins anchored on the cell surface and therefore require direct close juxtaposition of membranes to trigger a signal in responsive T cells. As a search strategy, CD8$^+$ T cells have developed a migratory lifestyle based on tunable control of their actomyosin cytoskeleton dynamics.[11,12] Thus, in contrast to, for example, stationary antibody-secreting plasma cells, CD8$^+$ T cells continuously undergo vigorous cell shape changes and cell body displacement to probe their environment and interrogate the surfaces of other cells for cognate pMHC. Chemoattractant receptors and integrins are essential factors to recruit blood-borne CD8$^+$ T-cell populations into target tissues. In addition, they play important roles in positioning CD8$^+$ T cells close to APCs or into specific microenvironments. The biochemical signals triggered by chemoattractant receptors, integrins, and the TCR itself are transformed within cells into mechanical work through the activity of the actomyosin cytoskeleton. This enables CD8$^+$ T cells to exert forces on neighboring cells and extracellular matrix (ECM) for cytotoxicity and migration. Since any nucleated cell can become infected, CD8$^+$ T cells have acquired the ability to infiltrate and inspect highly diverse tissue environments, which differ in topography, adhesion molecules, rigidity, and confinement.[13,14] Yet, in contrast to the "destroy" part, the regulation of actomyosin cytoskeleton for successful "search" is less comprehensively understood.

In this review, we will present a short overview over CD8$^+$ T-cell trafficking patterns before and after encountering cognate pMHC, highlighting their scanning behavior in lymphoid organs and their switch to NLT trafficking in response to external cues from chemoattractants and adhesion molecules. With this setting as the physiological background, we will outline some basic principles of how the dynamic actomyosin cytoskeleton network influences the efficacy of CD8$^+$ T-cell scanning, combining recent findings in cell biology with features of tissue anatomy. Finally, we will explore the hypothesis that some CD8$^+$ T cells may perform environmental scanning without external cues from chemoattractants and adhesion molecules, pending on the balance of actin regulators and its network architecture, for autonomous homeostatic surveillance of NLT.

1.1 | CD8$^+$ T-cell recirculation and pMHC search in lymphoid organs

Activation of CD8$^+$ T cells takes place in organized secondary lymphoid organs (SLO), such as lymph nodes (LN) and spleen. Although this task appears at first daunting given the low frequency of pMHC-specific T cells before clonal expansion (approximately 1 in 10$^{5-6}$ CD8$^+$ T cells[15]) and the large surface of the host organism, CD8$^+$ T cells are remarkably efficient in their search for rare cognate
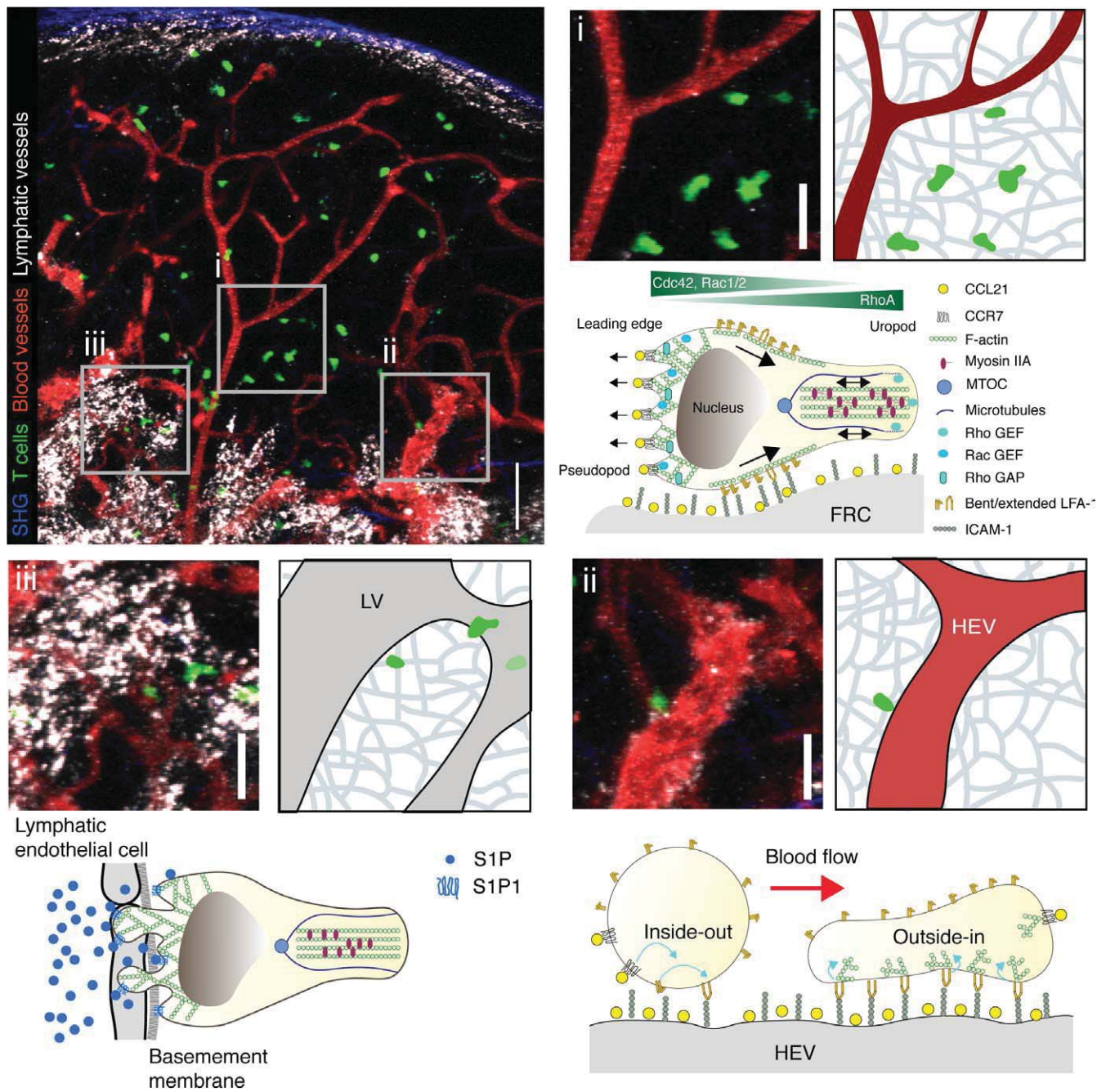
FIGURE 1 Regulation of T-cell trafficking in lymphoid tissues. Snapshots from intravital 2PM image sequence showing T cells (green) in the parenchyme of a mouse popliteal LN. (i-iii) High magnification snapshots with graphical reconstruction and cell dynamics. (i) Top. Paracortex with feeding arteriole. The FRC network is shown in gray. Bottom. Actomyosin dynamics in motile T cells. Polarized T cells generate Arp2/3-mediated branched F-actin networks at the leading edge, which leads to protrusions and, more importantly, generate a rearward cortical F-actin flow (indicated by black arrows). Through engagement with cytoplasmic tails of integrins and other transmembrane molecules, retrograde flow generates low-adhesive friction with the substrate for forward cell displacement. At the trailing edge, or uropod, F-actin cables are contracted through activity of non-muscle Myosin IIA. This helps to propel the nucleus as the biggest organelle through small pores. Arp2/3 activity is modulated by chemokine receptor signaling and requires Rac1/2 and Cdc42 activity. The most important Rac GEF for motility in T cells is DOCK2. Actomyosin contractility requires Rho-ROCK-mediated phosphorylation of the regulatory light chain of Myosin IIA and is facilitated by release of the Rho activator ArhGEF1 from depolymerizing microtubules. A RhoGAP, Myo9b, reduces Rho-GTP levels at the leading edge. (ii) Top. HEV with emigrating T cell. The FRC network is shown in gray. Bottom. Inside-out signaling (blue arrows) by CCR7 activation leads to rapid induction of an extended LFA-1 conformation on blood-borne T cells. Shear forces exerted by blood flow induce a high-affinity LFA-1 conformation, which in turn activates outside-in cytoskeletal rearrangement for crawling (blue arrows). (iii) Top. Medullary lymphatic vessel (LV). Bottom. A shallow S1P gradient is required to promote T-cell egress through DOCK2-dependent F-actin-filled protrusion formation. Scale bar overview, 50 μm and inserts, 20 μm

pMHC-presenting cells. In fact, there is a broad participation of virtually all available clones during adaptive immune responses.[15,16] Circulating naive CD62L^{+} CCR7^{+} CD44^{low} T cells are selectively recruited from the blood circulation into LN via high endothelial venules (HEV). Through the sequential activities of CD62L (L-selectin), CCR7 and LFA-1, which bind to their endothelial counterligands peripheral node addressin (PNAd), the homeostatic chemokines CCL19/CCL21, and ICAM-1/-2, respectively, T cells engage in a multistep sequence of selectin-mediated rolling and integrin-dependent arrest.[17] In brief, constitutive CD62L activity permits T-cell attachment to sulfated and sialylated sugar residues O-linked to the protein backbone of PNAd components. The resulting fast on-rate binding with short half-lives results in a rolling movement along the HEV surface, which presents heparan sulfate-bound CCL19 and CCL21[18] (Figure 1). CCL19 and CCL21 bind to CCR7 expressed on rolling T cells, which leads to LFA-1 activation for firm adhesion. The abrupt arrest through CCR7 "inside-out" signaling involves instantaneous transformation of the bent LFA-1 conformation found on blood-borne cells into an extended conformation for binding to ICAM molecules copresented on the HEV surface.[19] This process is mediated by the small GTPase Rap1 and is supported by mechanical shear forces exerted by continuous blood flow.[20] Active, ligand-engaged LFA-1 not only firmly arrests rolling cells but also triggers F-actin polymerization through "outside-in" signaling. This leads to T-cell flattening and crawling along the high endothelial cell surface. T-cell crawling requires ICAM-1 on the HEV surface, whereas ICAM-2 or VCAM-1 play no discernible role in this process.[21] LFA-1-ICAM-1-dependent T-cell crawling is reminiscent of the mesenchymal mode of cell motility, where integrin-triggered adhesion and F-actin formation permits cell body translocation along a 2D surface.[22] During crawling, T cells actively probe the endothelial cytoskeleton for permissive sites for transendothelial migration (TEM).[23] As a final step, T cells transmigrate through or in between HEV, which is facilitated by nuclear lobe protrusions.[24]

The observation that T cells are able to move away from an excess of luminal CCL21 is puzzling and the underlying molecular mechanism is not completely understood to date. It has been proposed that apical chemokine-mediated TEM is mediated by shear flow, in a process called chemorheotaxis.[25,26] Indeed, chemorheotaxis is readily recapitulated for T cells in in vitro flow chambers. When CCL21 is added on top of an endothelial cell layer, naive T cells rapidly transmigrate below the endothelial cell layer under flow.[27] In this setting, lack of shear flow impairs transmigration. Mechanistically, one conceivable scenario is that shear stress-extended integrins assemble intracellular activators of the small GTPase Cdc42. This in turn may lead to filopodia and podosome formation at the interface between crawling lymphocytes and the underlying endothelial surface to initiate TEM.

Alternatively, T cells crawling on endothelium have been shown to form protrusions that tap into intracellular pools of prestored chemokine inside vesicles within endothelial cells to promote transmigration.[28] Furthermore, high endothelial cells themselves may contribute to T-cell transmigration by acting as a shuttle to deliver their cargo pending on the "crowdedness" of the surrounding interstitium.[29] According to this model, endothelial cells sense the density of the LN interstitium and act as gatekeepers to maintain constant lymphocyte numbers inside the lymphoid tissue volume. One caveat to this proposal is that the LN volume itself is likely to change rapidly and frequently upon expansion or contraction of the surrounding tissue. Finally, an immobilized chemokine gradient around the HEV may contribute to the directed entry of lymphocytes across the HEV barrier.[30] This may further explain how transmigrated T cells are able to cross the endothelial basement membrane (BM) consisting of collagen IV (Col IV), laminins, and other ECM components.

Once inside lymphoid tissue, T cells rapidly polarize and move along CCL21- and ICAM-1-expressing fibroblastic reticular cells (FRCs) of the T-cell zone.[31] FRCs form a sponge-like loose 3D network that serves as a robust structural scaffold for T-cell surveillance of lymphoid tissue. FRC wrap themselves around secreted ECM fibers consisting of collagen I (Col I), Col IV, ERTR7, and laminins, which form the FRC conduit system for small molecule transport.[32] Intravital twophoton microscopy (2PM) imaging of the LN microenvironment in live, anesthetized mice has uncovered that migrating T cells adapt a polarized ameboid cell shape and follow a random FRC-guided motility pattern with high basal speeds of 12-15 μm/min, that is, more than one cell diameter per minute[33] (Minor differences in cell speeds between distinct laboratories may be in part due to technical considerations such as sampling frequency[34]). Thus, within the technical limitations of 2PM imaging, there is no discernable cluster-dependent motility or directed migration on a population level.[35] It is, therefore, common to observe fast migrating T cells moving in opposite directions within the same field of view, occasionally even crossing paths. In addition to speed and directionality, a useful parameter to quantify T-cell motility is the motility coefficient (MC), expressed in μm²/min. In physical terms, the MC corresponds to the 3D diffusion coefficient of a gas particle randomly moving in Brownian motion. In LNs, the MC of T cells can exceed 70 μm²/min, indicating a high ability to scan lymphoid tissue.

Fibroblastic reticular cells fulfill at least four functions for naive T-cell migration, survival and activation: First, these cells act as guidance cue for highly motile T cells by producing the promigratory chemokines CCL19 and CCL21, as well as ICAM-1. Of note, CCR7 ligands are not the only promigratory factor inside lymphoid tissue for T cells. Autotaxin-generated lysophosphatidic acid (LPA) stimulates T-cell motility by activating cell contractility.[36-38] Second, FRCs secrete IL-7, which together with CCL19 and CCL21 acts as survival-promoting cytokine.[39] Third, DCs use the FRC network as structural scaffold for DC attachment and scanning by T cells.[40] Finally, FRCs control immune responses through the release of prostaglandin E2, nitric oxide, and other mediators.[41]

The average dwell time of a T-cell population in a given mouse LN is approximately 6-8 hours and is determined by the balance of the egress-promoting sphingosine-1-phosphate receptor 1 (S1P1) and the retention-promoting CCR7.[42] Since S1P levels within lymphoid tissue are low in contrast to blood and lymph, recirculating T cells gradually increase S1P1 surface levels from very low levels right

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after entry into LN until reaching levels to become responsive to S1P in efferent lymphatic vessels. This returns cells via downstream LN and the thoracic duct into the blood circulation to ensure constant turnover of the TCR repertoire.[43] A detailed 2PM analysis of activated WT and S1P1^{-/-} effector T cells in reactive LN uncovered that both populations probed efferent lymphatic vessels but only WT T cells were able to cross into the lymphatic volume for egress.[44] This observation suggests that a shallow S1P gradient suffices to promote lymphocyte migration across the lymphatic vessel and its underlying BM (Figure 1). These data also lend support to the notion that despite vivid interstitial migration, T cells require local chemoattractants to cross dense tissue barriers such as BM in shear flow-free environments, a phenomenon explained by intrinsic F-actin network properties (see below).

Naive T-cell motility within lymphoid tissue has evolved to balance two opposing aims: On the one hand, T cells need to scan a large number of APC before their S1P1 surface levels start to promote egress via efferent lymphatic vessels.[45] On the other hand, T cells must be able to dwell long enough on individual DCs to integrate sufficient signals for activation. Recent work has confirmed that high intrinsic motility allows T cells to find rare DCs within large tissue volumes.[46-48] Yet, it also allows cells to detach from DCs presenting low amounts of cognate pMHC or low-affinity pMHC during the sampling phase of T-cell activation.[49] This is in line with pioneering work by Dustin and colleagues suggesting competitive roles between T-cell motility vs activation.[50] Nonetheless, T cells can arrest eventually for prolonged periods (h) even on "suboptimal" DCs once they have accumulated enough signals through short contacts.[51] To ensure prolonged LN dwell time under these circumstances, surface upregulation of CD69 has evolved to bind S1P1 in *cis* and induces its internalization. CD69 upregulation is induced both by specific TCR signaling on cognate T cells and, transiently and to lower surface levels, by type I IFN on all T cells.[52] Furthermore, T-cell activation leads to a transient loss in general migratory capacity of these cells, which is not well understood on a molecular level. This low motility presumably contributes to keep activated T cells trapped inside lymphoid tissue for full signal integration.[53,54]

1.2 | CD8^{+} T-cell activation and signal integration within lymphoid organs

Productive CD8^{+} T cell-DC interactions leading to expansion and effector differentiation require pMHC class I (signal 1) and costimulatory molecules CD80 and CD86 (signal 2) on DCs, while cytokines such as IL-2 and IL-12 provide signal 3. CD8^{+} T cells linearly integrate the input of these three signals to adapt their expansion according to the stimulatory strength.[55] On the other hand, early TCR signaling events such as ERK phosphorylation are often characterized by a digital, all or nothing response.[56] How can T cells adapt the level of input signal for an adequate response when initial signaling events are rather binary? One answer derives from intravital observation of CD8^{+} T-cell interactions with DCs presenting identical amounts of signal 2 and signal 3 but loaded with altered peptide ligands (APLs)

to grade signal 1 strength. In this experimental setting, early T cell-DC interaction dynamics are essentially identical, with an immediate arrest of CD8^{+} T cells irrespective of functional potency of the APL used for stimulation. Such a behavior correlates well with a digital behavior of early TCR signaling. In contrast, the total duration of these interactions was determined by the functional affinity of the peptide. Thus, CD8^{+} T cells detached from low potency cognate pMHC-presenting DCs earlier than from medium-or high potency-presenting DCs and constituted an early-wave CD8^{+} T_EFF population.[57] The affinity-dependent kinetics of T-cell detachment from DCs is therefore emerging as a mechanism to tune the extent of the signal integration in an analog manner while maintaining early binary responses.

T-cell detachment from DCs occurs efficiently in the lymphoid tissue microenvironment.[58] How this process unfolds under physiological conditions is currently unclear, also because T-cell detachment from APCs is difficult to reproduce in vitro. However, early evidence suggests that chemokines may be involved in this process.[50] A recent study by Bousso and colleagues has uncovered that once T cells had disengaged from DCs, they become temporarily unresponsive to additional pMHC on DCs in part owing to defective Ca-signaling responses.[59] This appears particularly relevant for low-affinity-primed T cells, whereas high-affinity-primed CD8^{+} T cells retain a longer window for additional signal integration by DCs. Whole LN reconstructions after light sheet fluorescent microscopy imaging have uncovered that recently primed CD8^{+} T cells upregulate the inflammatory chemokine receptor CXCR3 to accumulate at interfollicular regions of LN, where incoming DCs enter lymphoid tissue. There, these cells engage in productive secondary encounters for prolonged activation before becoming late-wave CD8^{+} T_EFF populations.[57] CXCR3-dependent relocalization to IRFs is also observed in central memory CD8^{+} T cells (T_CM), both in steady-state and shortly after viral infections. This process ensures a rapid encounter with incoming DCs and virus-capturing macrophages.[60,61] In sum, chemokines regulate both encounter with DCs at distinct locations within lymphoid tissues and perhaps also their detachment from these cells as prerequisite for egress.

1.3 | CD8^{+} T_EFF infiltration into NLTs

Following their activation in LN and spleen, CD8^{+} T_EFF undergo a switch from a SLO-targeted to a non-lymphoid tissue (NLT) trafficking pattern. This is important since infected cells are often localized at epithelial barrier tissues with restricted access for naive T cells. Thus, starting as early as 36-48 hours postpriming and peaking between 3 and 7 days postinfection, CD8^{+} T_EFF enter the blood circulation and invade multiple organs. Accordingly, T-cell activation leads to a reprogramming of chemoattractant receptor surface levels, such as de novo expression of inflammatory chemokine receptors CXCR3, CCR4, CCR9, and CCR10, as well as increased levels of the hyaluronan receptor CD44 and the integrins LFA-1 and VLA-4.[62] In parallel, T-cell activation leads to the expression of glycotransferases including enzymes for core2-O-glycan synthesis and fucosyltransferases

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IV and VII, which modify P-selectin glycoprotein ligand-1 (PSGL-1) to present ligands for endothelial selectins.[63,64] In sum, these changes promote the recruitment of CD8$^+$ T$_{EFF}$ into infected or injured organs. The anatomical location of the SLO where naive T cells first see their Ag and become activated determines the imprinting of their homing capacity toward mucosal vs skin-associated tissues. Thus, vitamin A derivatives from nutrient uptake imprint CCR9 and α4β7-mediated gut-homing capacity on T cells activated in gut-draining Peyer's patches and mesenteric LN. In turn, vitamin D derivatives induced by sunlight lead to selectin ligand synthesis and CCR10 expression, which imprint skin-homing capacities in activated T cells.[65,66]

Complementary to changes in CD8$^+$ T$_{EFF}$ homing molecules, tissue inflammation promotes increased adhesiveness of postcapillary venules through surface expression of P- and E-selectin, inflammatory chemokines such as the CXCR3 ligands CXCL9 and CXCL10, and the integrin ligands ICAM-1/VCAM-1, and MAdCAM-1 in mucosal tissues.[62] This is mediated by the local release of IL-1 and TNFα by tissue macrophages that act as sentinels through their PAMP receptors. In addition to recruitment to inflamed organs, CD8$^+$ T cells often accumulate in NLT irrespective of the presence of antigen or obvious inflammation in these organs. Such non-specific CD8$^+$ T$_{EFF}$ accumulation likely depends on the levels of systemically circulating IL-1 and TNFα that increase adhesion receptors and chemokines on all postcapillary EC surfaces.[67] In addition, skin and mucosal tissues, which are constantly exposed to microbiota or microbiota-derived molecules, display constitutive adhesiveness of their postcapillary venules. As example, dermal postcapillary venules express E-selectin on their surface, which permits baseline adhesion of rolling leukocytes in the absence of overt inflammation.[68] In sum, homing of blood-borne T$_N$ and T$_{EFF}$ is critically dependent on adhesion molecules and chemoattractant receptors that respond to organ-specific complementary receptors on endothelial cell surfaces, in a manner akin to a combinatorial area code.[69]

Once accumulated in their target organ, the presence of cognate Ag boosts CD8$^+$ T$_{EFF}$ numbers at sites of infection and contributes to their long-term retention.[70] In skin viral infections, CD8$^+$ T$_{EFF}$ use CXCR3 to accumulate at viral foci that release the ligands CXCL9 and CXCL10.[71,72] In contrast to the directed migration of neutrophils to sites of tissue injury or bacterial deposition,[73,74] epidermal CD8$^+$ T cells achieve this by a subtle modulation of their migration angle distribution toward the source of CXCR3 ligands.[72] Similarly, CXCR3 facilitates dermal CD8$^+$ T$_{EFF}$ migration to the epidermis.[75] In adjuvant-inflamed skin cells, the αv integrin contributes to tissue scanning of dermal effector CD4$^+$ T cells.[76] Thus, chemokines and integrins regulate the migration of T$_{EFF}$ in acutely inflamed tissue, at least in some settings.

1.4 | Dynamic tissue surveillance by memory CD8$^+$ T cells

After the clearance of a viral infection, most CD8$^+$ T$_{EFF}$ die through apoptosis in the contraction phase. The remaining memory cells are classified into several subsets. Central memory CD8$^+$ T cells (T$_{CM}$) continue to patrol secondary lymphoid organs similar to naive T cells. As outlined above, these cells preferentially localize or become rapidly recruited after infection to interfollicular regions, where Ag is first transported to by incoming migratory DCs. A recent study has uncovered that T$_{CM}$ efficiently infiltrate NLT without the need to become first activated in LNs or spleen.[77] This function was mostly attributed to another memory CD8$^+$ T-cell subset, the effector memory T cells (T$_{EM}$). Originally, T$_{EM}$ had been defined in human blood samples as CD45RO$^+$ memory T cells with low CCR7 and CD62L levels.[78] CD62L$^-$ CCR7$^-$ CD44^{high} CD8$^+$ T$_{EM}$ are also readily identifiable in mice. Yet, their precise function during recall responses is incompletely understood, and CD8$^+$ T$_{EM}$ may actually comprise a variety of distinct subpopulations. In this context, recent work has identified three types of effector/memory CD8$^+$ T cells, which are characterized by varying degrees of CX3CR1 expression. CX3CR1$^+$ CD8$^+$ T cells appear around day 6 postviral infection and can be divided into CX3CR1^{int} and CX3CR1^{high} subsets. Only the CX3CR1^{int} population was effectual in peripheral tissue surveillance.[79] The precise function of CX3CR1 and its ligand CX3CL1 is not clear, since the absence of this receptor does not prevent the formation of the three effector/memory T-cell subsets.[79]

A recently described memory CD8$^+$ T-cell subset derives from tissue-infiltrating KLRG-1^{low} CD8$^+$ T$_{EFF}$. Instructed by tissue-specific cues, such as TGF-β and IL-15 in skin, these cells become resident in the target tissue after pathogen clearance and hence are called tissue-resident memory T cells (T$_{RM}$). Many T$_{RM}$ upregulate CD69 and CD103 in epithelial-rich tissue.[80-83] In contrast to other memory T cell subsets, T$_{RM}$ do not enter blood or lymph in large numbers, but remain inside their tissue of residence as a self-maintaining population for prolonged periods of time. While T$_{RM}$ were first described in skin following local viral infections, they have now been uncovered in virtually all organs, including gut, salivary glands, liver, lung, genitourinary tract, central nervous system, and SLO.[84,85] Their function is best understood in skin and the genitourinary tract. Following a re-exposure to the original pathogen-derived pMHC, T$_{RM}$ rapidly secrete IFNγ and other cytokines. This in turn activates tissue macrophages and other cells of the innate immune system and provokes a tissue-wide alert status.[81,83] Thus, the presence of T$_{RM}$ in barrier tissue locally reverses the paradigm that innate immunity precedes the onset of adaptive immune reactions. A second consequence is the rapid recruitment of circulating memory T cells to contain expanding microbes.[82] Furthermore, CD8$^+$ T cells with a T$_{RM}$ phenotype have been identified in tumor tissue and their presence strongly correlates with a good prognosis.[86]

Given their infiltration into a wide range of distinct tissues, CD8$^+$ T$_{RM}$ constitute an ideal tool to address how cells from the same starting pool of naive T cells adapt to specific microenvironments. Intravital imaging of distinct T$_{RM}$ populations in epidermis, liver, and genitourinary tracts has provided evidence of a continuous scanning behavior, even in the absence of replicating pathogens.[87-90] This reflects their dependence on recognizing membrane-bound pMHC in order to fulfill their function of host cell surveillance. However, T$_{RM}$

speeds and MC as a proxy for scanning efficacy differ vastly within different tissues, which may reflect physical constraints of their microenvironments. In the epidermis consisting of tightly packed layers of keratinocytes, T_{RM} speeds become as low as 1-2 μm/min, which restrains their dissemination away from the site of initial recruitment.[87,88] In the connective tissue of dermis, T_{RM} speeds increase to 6-8 μm/min.[91] How do motile T_{RM} achieve their long residence time in tissue containing blood and lymphatic vessels without being flushed away or migrate out of the tissue? A key step in establishing tissue residency is the downregulation of the transcription factor KLF2, which promotes expression of S1P1 and CCR7.[92] Accordingly, forced expression of S1P1 counteracts the establishment of long-term resident CD8^{+} T-cell populations. Therefore, unresponsiveness to S1P and potentially CCL21 secreted by lymphatic vessels helps to maintain T_{RM} within tissue. In skin epidermis, T_{RM} typically localize close to the dermal-epidermal junction both in mouse and human tissue sections.[93] Their retention in this part of the basal keratinocyte layer may be imposed by local chemoattractants or specific adhesive interaction to prevent them from exiting the epidermis by the constant renewal of upper layers from proliferating basal keratinocytes. In support of this, CD103 (αE integrin) binds E-Cadherin on epithelial cells and contributes to local T_{RM} retention but does not influence migration speeds in epidermis.[75] Along the same line, increased LFA-1 levels are required for long-term retention of sinusoid-patrolling T_{RM} in liver.[90] Taken together, regulation of adhesiveness to the surrounding epithelium and low surface levels of receptors for egress-promoting chemoattractants contribute to T_{RM} tissue residency.

2 | PART 2. REGULATION OF CD8^{+} T-CELL MOTILITY BY THE ACTOMYOSIN CYTOSKELETON

Integrins and chemoattractant receptors trigger intracellular signaling pathways that lead to changes in shape and dynamic behavior. Eukaryotic cells typically express four types of protein families that determine their shape and biophysical properties: the microtubular network, intermediate filaments, septins and the actomyosin network. Each of these scaffolds plays important roles for CD8^{+} T-cell function. Thus, microtubules in effector CD8^{+} T cells deliver cytotoxic granules to target cells.[2] Furthermore, depletion of the microtubular network increases active Rho-GTP levels, which in turn induces contraction and protrusion retraction.[94] The role for intermediate filaments such as vimentin in CD8^{+} T cells is not well studied, although these filaments contribute to the rigidity of circulating lymphocytes.[95] Septins consist of 13 members, which form hetero-oligomeric complexes and higher order structures such as rings.[96] In CD8^{+} T cells, septins are required for persistent motility and proliferation.[97,98] Here, we focus on the actomyosin cytoskeleton, arguably the most critical network to convert biochemical signals into mechanical work for CD8^{+} T-cell shape and displacement. F-actin is composed of polymerized G-actin monomers. While the addition of G-actin monomers to pre-existing barbed F-actin ends

occurs rapidly, the initial polymerization of individual G-actin beyond a dimer/trimer requires nucleation factors.[99] Prominent nucleation factors of CD8^{+} T cells are the heptameric Arp2/3 complex and the formins mDia1 and mDia2. The Arp2/3 complex becomes activated by Cdc42-GTP and Rac1/Rac2-GTP via the nucleation-promoting factors WASP and SCAR/WAVE, respectively, to create new F-actin filaments on preformed F-actin filaments in a 70° angle. Thus, Arp2/3-mediated nucleation creates a branched F-actin network and is preferentially generated at the leading edge of migrating CD8^{+} T cells to form pseudopods (Figure 1). Pseudopods are evolutionary conserved actin-filled protrusions characteristic of ameboid cell motility and require both WASP and SCAR/WAVE.[100] In high-resolution microscopy, pseudopods actually appear as interleaved microlammelipodia.[101] The precise actin network architecture is determined by F-actin-binding proteins. ENA/VASP proteins protect barbed ends from capping protein and thus contribute to filament elongation. Similarly, the actin nucleation factor mDia protects growing actin filaments, whereas cofilin destabilizes existing filaments to promote actin turnover.[102] The Arp2/3 inhibitor Arpin and coronin also limit Arp2/3-mediated F-actin network expansion.[103-105] Thus, branched F-actin networks are highly dynamic with constant filament turnover.

In contrast to Arp2/3, the nucleation factors mDia1/2, also known as formins, mediate actin nucleation to create linear F-actin filaments. Upon phosphorylation of the regulatory myosin light chain (MLC), non-muscle Myosin II assembles into bipolar filaments that enable linear F-actin filaments to move in an antiparallel manner, leading to contraction.[106] Myosin-mediated contractility of the cortical F-actin network in resting cells results in the spherical cell shape observed in the absence of extracellular polarity-inducing agents. Upon induction of polarity (eg, by addition of chemokines), pMLC accumulates at the trailing edge of migrating cells, where it mediates uropod contractility (Figure 1). Contractility serves several critical functions. It is required to detach the trailing edge containing LFA-1 and other adhesion molecules such as CD44 away from sites of adhesion.[107] In addition, uropod forces are required to push the nucleus through narrow pores in vitro and in vivo. Thus, inhibition of uropod contractility using the Myosin II inhibitor blebbistatin or the ROCK inhibitor Y27632 leads to a phenotype where the leading edge still migrates toward a chemotactic source, whereas the nucleus becomes physically stuck.[108] Uropod contractility also leads to an anterograde flow of cytoplasm. Depending on the anchorage of the leading edge cortical actomyosin cytoskeleton to the plasma membrane, the resulting hydrostatic pressure may lead the detachment of the plasma membrane from the underlying cytoskeleton.[109] This leads to the formation of membrane blebs, which are rapidly refilled with F-actin.[110] Bleb formation has been reported for a variety of cell types in vivo, such as during zebrafish gastrulation. There, bleb formation contributes to successful steering of cell migration.[111,112] Blebs do not appear to play a role during trafficking of naive T cells inside lymphoid tissue, where F-actin-filled pseudopod protrusions are predominant.[113] Bleb formation may in turn constitute one of the migration strategies acquired by CD8^{+} T cells in NLT. Yet, it remains

unclear whether CD8^{+} T cells in NLT are able to develop sufficient contractility required for bleb formation. This is experimentally challenging to address, since blebs constitute transient structures difficult to resolve by intravital imaging.

The branched F-actin network is constantly remodeled by factors such as glial maturation factor (GMF). GMF disassembles Arp2/3-containing branched junctions without severing actin filaments, which may render these filaments amenable to Myosin II-mediated contraction at the uropod. Accordingly, GMF isoforms are highly expressed in CD8^{+} T cells throughout all stages of immune responses (www.immgen.org). Finally, Myosin II activity contributes to disassembly of actin filaments for cell-scale actin treadmilling.[114] From these properties, unexpected features can emerge such as the universal coupling of cell speed and the straightness of movement: the faster cells migrate, the higher is their directionality persistence. High migratory speed stabilizes cell directionality by transporting intracellular polarity cues, such as Myosin II, to the trailing edge through fast rearward F-actin flow.[115]

An intrinsic key feature of F-actin polymerization was recently uncovered by high-resolution imaging of branched F-actin angle distribution at the leading edge.[116] With increasing membrane load, which may correlate with encountering a dense barrier during
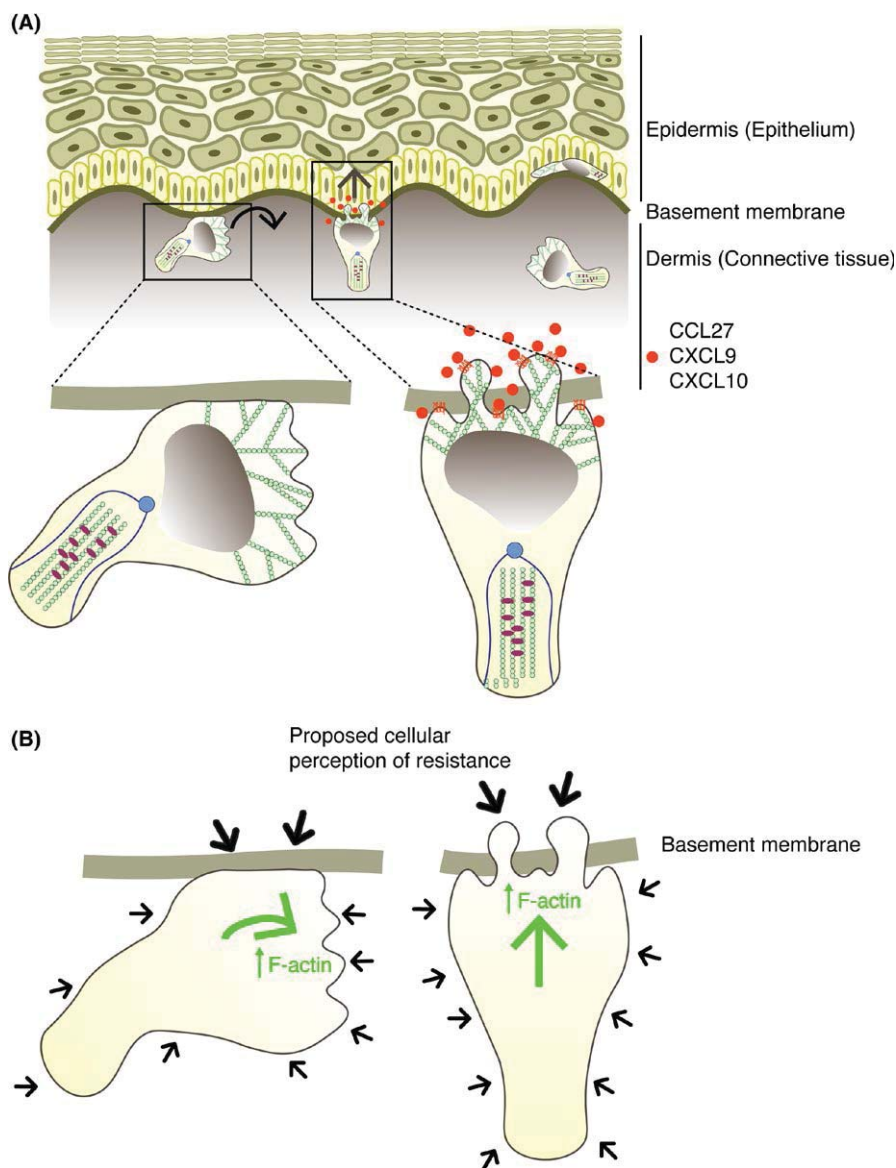
FIGURE 2 Role of chemokines in barrier crossing and perception of resistance by T cells in non-lymphoid tissue. A, As an example, CD8^{+} T$_{EFF}$ migration in skin is pictured. In order for dermal CD8^{+} T$_{EFF}$ to accumulate in the epidermis, they have to cross the basement membrane (BM) separating dermis from epidermis. In the absence of attraction by chemokines released from keratinocytes, the leading-edge F-actin dynamics are wired to form leading edge protrusions away from dense barriers such as the ECM of a BM. This is in part because fast-growing F-actin filaments accumulate preferentially where the plasma membrane experiences less resistance. When stimulated by chemokines, chemokine receptors on CD8^{+} T$_{EFF}$ lead to local pikes of protrusive F-actin activity to insert pseudopods through permissive gaps of the BM ECM mesh. The nucleus likely acts as a ruler to avoid nuclear rupture, which would occur when cells try to pass through non-permissive gaps. B, Proposed perception of resistance to generate F-actin protrusions as indicated by arrows from a migratory T-cell perspective

migration, leading edge F-actin branching angles became broader with shorter fragments. In contrast, where membrane load was decreased, expanding actin filaments growing perpendicular to the plasma membrane outran filaments growing at steeper angles. When reaching the plasma membrane, fast perpendicular filaments become protected from capping proteins by factors such as VASP/ENA and formins.[116] This leads to faster membrane protrusion speeds and, by force coupling, efficient cell body translocation. This model provides an explanation for the remarkable mechanosensitivity displayed by leukocytes during their migration through 3D collagen matrices, where these cells choose the path of least resistance[117] (Figure 2).

Pseudopod protrusion speeds alone do not determine the path of tissue-infiltrating T cells. Rather, the nucleus as the biggest organelle is likely to play a central role for T-cell decision taking on which path to follow in geometrically complex environments. Thus, it has emerged in recent years that nuclear deformability determines the physical limitations of cell migration. The stiffness of the nuclear envelope is determined by the expression of the structural proteins lamin A and lamin C.[118] In leukocytes, their expression is low, indicating a relatively soft nuclear envelope in line with their motile lifestyle.[119] When cancer cells are forced through narrow pores in microchannel systems, their nuclear membrane may rupture, leading to the recruitment of the ESCRT repair mechanism.[120,121] It is conceivable that similar mechanisms maintain T-cell nuclear integrity intact during prolonged tissue surveillance, in particular in tight epithelial environments. Maintaining nuclear integrity also imposes a physical limit to T-cell migration in the absence of proteolytic degradation of the ECM. For T-cell blasts, the minimum pore size is around 4 μm^2, which correlates to a circle with a diameter of approximately 2.5 μm.[122] Taken together, leading edge F-actin dynamics and nuclear restriction are important parameters for path finding in complex environment. How is cell body displacement accomplished by dynamic F-actin remodeling and contraction?

2.1 | F-actin network dynamics and T-cell motility

Mesenchymal cell migration (eg, fibroblast migration) is achieved by F-actin protrusions at the lamellipodium to push the membrane forward, followed by new integrin-mediated focal adhesions and trailing edge contraction.[123,124] In this migration mode, elevated substrate adhesiveness actually stalls migration speeds owing to the necessity to detach focal adhesions. Similarly, T-cell blasts placed on a 2D surface (which has been the experimental standard over the last decades) require integrins, typically LFA-1, and an immobilized integrin ligand, such as ICAM-1, on the surface for cell displacement.[125] This is because integrins are required for cell attachment on the surface in 2D systems, as well as for outside-in signaling to the actomyosin cytoskeleton and for force transmission from retrograde F-actin flow (Figure 1). Such a motility mode may partially reflect physiological T-cell migration on endothelial surfaces under flow, although in the latter case shear forces contribute to integrin activation.[20]

In contrast, ameboid motility in 3D environments does not involve strong substrate anchoring. In general, there are four modes of focal adhesion-free migration: (a) swimming migration in blebbing cells, (b) cell substrate intercalation by insertion of protrusions into preformed or deformable gaps of the environment, (c) chimneying by exerting force to ECM or adjacent cells perpendicular to the direction of migration in order to keep the cell body in place, and (d), flow-friction-driven force transmission by retrograde cortical actin flow.[126] Recent work has shown that the latter mode is used by naive T cells for migration under in vitro and in vivo confinement. When these T cells are placed under confinement, for example, by squeezing these cells between a coated plate and an agarose layer, on ICAM-1 coated plates in the presence of CCL19 or CCL21, these
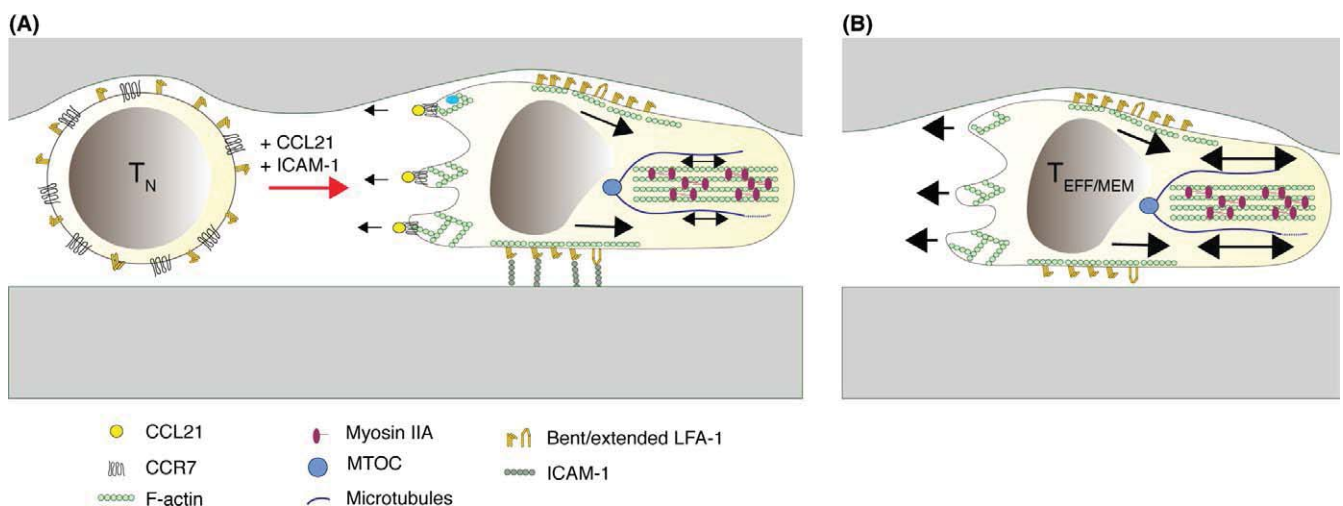
FIGURE 3 Models of extrinsic vs intrinsic regulation of cell motility. A, Naive CD8$^+$ T cells (T_N) under confinement are immotile unless a chemokine is provided to induce polarization and rearward F-actin flow. Integrin ligands promote cell body translocation through weak interactions, without inducing adhesion. B, CD8$^+$ T_{EFF}, and T_{MEM} can in principle tune their intrinsic F-actin treadmilling and/or contractility independent of extrinsic cues for spontaneous motility. The precise contribution of intrinsic regulation during CD8$^+$-mediated immune surveillance remains unknown

cells show remarkable high migration speeds reminiscent of values observed in lymphoid tissue (Figures 1 and 3A). Under these conditions, LFA-1 becomes dispensable for cell attachment since cells are physically forced into contact with the environment. High-resolution imaging of F-actin dynamics in confined T cells exposed to CCR7 ligands show constant F-actin polymerization at their leading edge. Many newly formed F-actin filaments are rapidly pushed toward the back of the cell, presumably owing to deformation resistance of the leading edge plasma membrane. This results in fast F-actin retrograde flow tuned by the strength of chemokine receptor signaling. The speed of the rearward F-actin flow determines the morphology of the cell, with high flow speeds corresponding to elongated shapes.[113] In the reductionist under agarose setting, LFA-1 transmits weak frictional interactions to the substrate but does not itself contribute to outside-in F-actin polymerization. Thus, LFA-1 becomes engaged by retrograde F-actin flow to weakly interact with its ligand ICAM-1.[127] Through this clutch function, rearward F-actin flow slows down and becomes transformed into forward movement, which is tuned by ICAM-1 density. In contrast to the mesenchymal mode of motility, high substrate adhesiveness does not induce lymphocyte arrest because no focal adhesions are formed.

In vivo morphometric analysis of CCR7$^{-/-}$, LFA-1$^{-/-}$ and double-deficient T cells migrating on the FRC network of lymphoid tissue have confirmed key aspects of the proposed model, such as cell elongation in the absence of LFA-1 and cell shortening in the absence of CCR7. In addition, while absence of either molecule resulted in a speed decrease, double-deficient T cells showed a more pronounced loss of cell body translocation. These data clearly demonstrate a complementary input by both modules for cell translocation in interstitium, in contrast to their sequential engagement during extravasation.[113] In sum, the mode of motility in naive T cells within lymphoid tissue is consistent with a cortical actin flow model proposed by Bray and White[128] and permits a continuous sliding mode of T cells while limiting their adhesive interactions with the surrounding environment. One of the implications of this model is that selective LFA-1 engagement may be restricted to encounters with cognate pMHC-presenting DCs, as has been suggested by in vitro studies of the IS and the reduced interaction time of CD8$^+$ T cells with ICAM-1-deficient DCs in vivo.[129,130] Yet, a recent study has challenged the concept that LFA-1-ICAM-1 interactions are required for T-cell arrest on pMHC-presenting DCs during the first hours of their encounters.[131] Thus, the precise role of LFA-1 during T-cell activation in vivo and IS formation is still not well understood.

2.2 | Regulators of the actomyosin cytoskeleton in CD8$^+$ T cells

Biochemical information triggered by chemokines and other mediators is transformed by leukocytes into mechanical work through the activity of the actomyosin cytoskeleton. Which factors in turn orchestrate the actomyosin network assembly and disassembly? As in all eukaryotic cells, members of the small GTPase family, RhoA, Rac and Cdc42, play central roles in T cells. These proteins cycle between GDP-bound inactive states and GTP-loaded active states, in which these molecules interact with downstream effector molecules. Rac1 and Rac2, both of which are expressed in leukocytes, activate Arp2/3 complexes via SCAR/WAVE complexes. Lack of either Rac1 or Rac2 only partially reduces speeds of naive T cells within lymphoid tissue, suggesting largely overlapping functions of these isoforms for optimal chemokine-triggered F-actin generation.[132] Accordingly, in lymphoid tissue of plt/plt mice lacking the promigratory factors CCL19 and CCL21, T-cell speeds decrease to a similar level between WT T cells and T cells lacking either Rac1 or Rac2. This suggests that under suboptimal migration conditions, one Rac isoform suffices to transmit signals for cell motility. In contrast, lack of both Rac1 and Rac2 precipitates a strong loss in T-cell motility.[132]

As outlined above, Cdc42 uses WASP complexes for Arp2/3 activation. Detailed FRET-based reporter activity measurements have uncovered that while both Rac and Cdc42 activity localizes to the leading edge of migrating leukocytes, local Cdc42 signals precede Rac activation before cell turning.[133] Finally, in contrast to fibroblasts and other mesenchymal cells, Rho activity is restricted to the trailing edge and mediates uropod contractility via ROCK-mediated MLC phosphorylation (Figure 1). In resting cells, such as in lymphocytes freshly isolated from blood, levels of active, GTP-loaded Rac1/2 and Cdc42 are low. RhoA-ROCK-Myosin II activity is also low but likely to be present, indicated by the spherical shape of cells under these conditions (most cells adapt a spherical shape in suspension owing to their intrinsic cortical actomyosin cytoskeleton contractility).

2.3 | GEFs and GAPs are critical regulators of actomyosin dynamics

After stimulation with chemoattractants, CD8$^+$ T cells acquire a polarized shape with a leading edge and a trailing edge, and separated activities of small GTPases. Small GTPases themselves are activated by enzymes, which help to load GTP in place of GDP into the binding pocket to activate small GTPases, so called guanine exchange factors (GEFs). The approximately 80 GEFs identified in mouse and human genome do so by stabilizing the nucleotide-free form of small GTPases, which then allows the replacement of GDP by the more abundant GTP. There are several families of GEFs with distinct active domains.[134] The largest subgroup of GEFs are the Dbl homology (DH)-containing proteins including Vav, which is well characterized for its role during TCR signaling. Yet, the involvement of Vav proteins in primary CD8$^+$ T-cell motility is minor.[135] Another member, Tiam1, participates in CXCL12-induced T-cell migration through Rac activation but its role in CCR7-mediated migration and in vivo trafficking has not been investigated in depth.[136]

A second family is constituted by dedicator of cytokinesis (DOCK) proteins. DOCK family members express the DOCK homology region (DHR) 2 as catalytically active domain. While DOCK1 is expressed in most non-hematopoietic cell types, DOCK2 is a hematopoietically expressed Rac1/Rac2 GEF. Genetic deletion of DOCK2 results in strongly diminished F-actin polymerization after chemokine stimulation and leads to a phenocopy of Rac1 and Rac2-deficient

T cells, that is, virtually abolished mobility of T cells in lymphoid tissue.[132,137,138] DOCK2 is therefore the main Rac1/Rac2 GEF implicated in chemokine-induced Arp2/3 activation in T cells. DOCK2 also leads to Rac activation downstream the S1P1, which delays egress of DOCK2-deficient T cells from lymphoid tissue.[138]

Intravital imaging of DOCK2-deficient T cells in lymphoid tissue has identified two roles accomplished by DOCK2-Rac-axis during immune responses. First, as predicted by modeling, lack of motility strongly reduces the likelihood of DOCK2$^{-/-}$ T cell encounters with rare DCs distributed throughout lymphoid tissue. Second, DOCK2-driven motility is required to allow T cells to find and cluster around rare "optimal" DC, that is, DCs with high levels of cognate pMHC. DOCK2 does so by promoting T-cell detachment from DCs with low levels of cognate pMHC.[49] This supports the notion that high interstitial motility of T cells has not only evolved to search for antigen but serves also to limit T-cell activation: only signals, which induce a strong stop signal, or which are repeatedly encountered over the course of several hours, are licensed to give rise to T-cell activation. Thus, chemokines regulate T-cell responses not only by guiding to APC but additionally by maintaining high basal motility as quality control for the strength of the activatory signal.

DOCK2 also regulates F-actin dynamics at the IS of activated T cells.[139] There, the lipid-binding DHR1 domain of DOCK2 binds phosphoinositide-3,4,5-phosphate (PIP3) of the peripheral supramolecular activation cluster to drive centripetal F-actin polymerization. This leads to the transportation of TCR to the central supramolecular activation cluster and exerts forces that may help the TCR differentiate between low- and high-affinity ligands.[6,140] As a consequence, Arp2/3-dependent F-actin polymerization at the IS is strongly reduced in DOCK2-deficient T cells. This correlates with defective T-cell activation, despite the fact that most downstream signaling events except Rac activation remain intact.[141] Finally, DOCK2 functions in T-cell activation beyond facilitating DC encounter and direct TCR signaling. The homeostatic chemokine CCL21 acts as a costimulatory molecule during naive T-cell activation, which is in most part mediated by Rac-driven ERK activation.[142] The importance of DOCK2 for the functioning of the immune system is reflected in the early-onset severe immunodeficiency in humans lacking DOCK2 expression.[143]

DOCK8 is a Cdc42 GEF and allows DCs to navigate geometrically complex environments.[144] CD8$^+$ T cells lacking DOCK8 show comparable expansion during an adaptive immune response, but their memory populations contract rapidly.[145,146] Human CD8$^+$ T cells with decreased or absent DOCK8 expression owing to genetic mutations undergo cell death in collagen matrix owing to disintegration of cell shape, a process termed cytothripsis.[147] In line with disturbed T-cell trafficking, inhibition of DOCK8-Cdc42-mediated migration ameliorated the disease course in a mouse model of multiple sclerosis.[148]

ArhGEF1 (also known as p115GEF or lsc) is a Rho GEF highly expressed in naive and activated T cells. In its absence, Rho-GTP loading and migration are severely impaired in response to chemoattractants.[149] In polarized T cell lines, ArhGEF1 is sequestered by the microtubule network at the trailing edge. Microtubule disassembly results in the release of this factor, leading to activation of Rho. This in turn triggers ROCK-dependent MLC phosphorylation and uropod contractility[94] (Figure 1). In addition to the examples given above, T cells express more than 20 GEFs, and their expression patterns often change during activation (www.immgen.org). Yet, the roles of most members of the DOCK and Dbl families for the in vivo function of CD8$^+$ T cells remain largely unknown to date.

Activated small GTPases cleave GTP to GDP to return to the resting state, but do so with relatively slow kinetics. GTPase-activating proteins (GAPs) bind to the active, GTP-bound forms of Rho, Rac, and Cdc42. GAP binding catalyzes the intrinsic GTPase activity to convert small GTPases to the GDP-bound, inactive form. Similar to GEFs, there are more than 60 GAPs found in mouse and human genome, thus outnumbering the number of small GTPases. The catalytic domain is conserved between GAPs, pointing to a common evolutionary origin. Similar to GEFs, CD8$^+$ T cells express more than 20 identified GAP members (www.immgen.org), and the physiological function of most of these factors is unknown to date. Of note, in an unbiased genetic screen to identify factors facilitating intratumoral CD8$^+$ T-cell accumulation in a mouse model of melanoma, the authors identified the Rho GAP ArhGAP5 as an inhibitor of CD8$^+$ T$_{EFF}$ infiltration.[150] Yet, the mechanism of action has thus far not been described, and may conceivably involve changes in T-cell activation as well as altered ability to respond to chemoattractants.

Local suppression of small GTPase activity by GAPs serves a similar function as local activation, namely to compartmentalize pools of active Rac/Cdc42 and Rho to the leading and trailing edge of polarized cells, respectively. As an example, Myosin IXB (Myo9b) is an F-actin-binding cytoskeletal motor protein with a RhoGAP activity as "cargo,"[151] which accumulates at the leading edge of polarized macrophages and DCs.[152] In these cell types, Myo9b deficiency leads to increased steady-state Rho-GTP levels and a contracted cell phenotype that resulted in impaired migration in vitro and in vivo. Absence of Myo9b in T cells also resulted in increased steady-state Rho-GTP levels, reduced in vitro migration toward homeostatic chemokines and lower LN homing in vivo.[91] Despite these defects, Myo9b$^{-/-}$ CD8$^+$ T cells showed similar clonal expansion and effector differentiation in spleen and LN as their WT counterparts during DC- or virus-triggered inflammation. In contrast, Myo9b$^{-/-}$ effector CD8$^+$ T cells failed to efficiently seed NLTs and to protect hosts from skin infection. This phenotype correlated with a strongly reduced ability to cross dense ECM barriers in vitro.[91] Taken together, Myo9b-dependent repression of Rho activity at the leading edge has evolved to enable effector T cells to negotiate tissue barriers, in particular those formed perpendicular to their migration path. Specifically, T-cell seeding of non-lymphoid epithelial tissues that are separated by a dense BM from underlying connective tissue is critically dependent on carefully balanced Rho activity and for the establishment of protective CD8$^+$ T$_{RM}$ populations.[91] These findings also highlight the impact of tissue architecture and properties on T-cell infiltration and surveillance (Figure 2).

The Rho regulator Fam65b is highly expressed in naive T cells, where it contributes to the maintenance of low steady-state Rho-GTP levels akin to Myo9b. Yet, in contrast to Myo9b, Fam65b does not

specifically accumulate at the leading edge of polarized T cells but is uniformly distributed along the plasma membrane.[153] There, Fam65b sequesters Rho and prevents its GTP-loading. Upon chemokine-triggered Ser/Thr phosphorylation, Fam65b transiently detaches from the plasma membrane to permit Rho-GTP formation.[153] A salient feature of CD8^{+} T_{RM} cells is their decrease in expression levels of Fam65b.[154] This suggests that low Fam65b levels in T_{RM} may lead to permanently increased Rho-GTP levels, and thus increased contractility of their cortical actomyosin cytoskeleton. This may reflect an adaptation to more dense microenvironments of NLT as compared to the loose fibroblastic scaffold found in SLO. Despite these few examples, the role for most of the GAPs during global positioning through integrin regulation and migration control remain unaddressed to date.

2.4 | Beyond GEF and GAPs: additional regulators of T-cell motility

Along with recent discoveries on GEF and GAP function for T-cell immune surveillance, a number of unexpected regulators of chemokine-triggered T-cell motility have emerged in recent years. While it is beyond the scope of this review to provide a comprehensive overview, we present a few notable examples. Studies on the actin-binding protein myosin 1G (Myo1g) have uncovered a link between T-cell motility patterns and activation phenotype. Myo1g accumulates at sites adjacent to sites where the T-cell plasma membrane encounters obstacles, and contributes to the formation of a new F-actin protrusion next to such obstacles.[155] This confers to T cells the ability to meander and efficiently scan their surrounding environment. In turn, Myo1g-deficient T cells display a more directional motility inside lymphoid tissue as compared to WT T cells. 2PM imaging showed that the decreased meandering ability shortened the average duration of individual T cell-DC contacts. This decrease in contact time was compensated when DCs were abundant within lymphoid tissue, since CD8^{+} T cells remained able to integrate sufficient information for full activation. In contrast, under conditions of low DC frequency, this aberrant behavior resulted in impaired T-cell activation.[155] This observation provides further evidence that the migratory behavior of T cells is finely tuned to balance efficient tissue scanning with sufficient signal integration for activation.

The Ser/Thr kinase WNK1 is best known for its role in regulating salt homeostasis in kidney. Unexpectedly, in T cells, WNK1 acts as a negative regulator of LFA-1 activity while positively regulating chemotaxis via the kinases OXSR1 and STK39 and the ion transporter SLC12A2. Thus, lack of WNK1 in primary T cells resulted in an hyperadhesive, hypomotile phenotype with a strongly decreased ability to inspect large tissue volumes.[156]

Regulators of the "large" GTPases, that is, the Gαi subunits of GPCRs, in particular RGS1, regulate T-cell migration in vivo.[157] These proteins do so by accelerating the intrinsic GTPase activity of GTP-bound Gαi to fine-tune responsiveness to chemotactic signals. In contrast to Fam65b, RGS1 is specifically upregulated in T_{RM}. While its in vivo function remains incompletely understood, RGS1 may help to suppress signaling by tissue egress-promoting factors,

in particular S1P or in case of epidermis, CCL21. Thus, lack of RGS1 may contribute to facilitate long-term tissue residency in these cell populations by regulating responsiveness to chemoattractants, in addition to decreased S1P1 and CCR7 levels.

2.5 | CD8^{+} T-cell membrane properties and exertion of forces

In recent years, it has become increasingly clear that regulation of the F-actin network affects the biophysical properties of T-cell membranes. One important readout is the plasma membrane tension, which is related to the force needed to deform a membrane. Owing to the task of CD8^{+} T cells to interact with DCs and scan the surfaces of other cells, control of membrane tension is critical. Membrane tension is influenced by three components: first, "in-plane" tension describes the tension between lipids of the membrane bilayer and is influenced in part by osmotic pressure. It is generally assumed that local rises of in-plane tension become distributed within milliseconds throughout the plasma membrane.[158,159] However, this has been put into question by a recent study.[160] Second, membrane-to-cortex attachment (MCA), or membrane-cytoskeleton adhesion, describes the anchorage of the cortical actomyosin cytoskeleton through adaptors for transmembrane proteins such as phosphorylated Ezrin/radixin/moesin (pERM) or protein binding lipids such as PIP2 of the inner leaflet of the plasma membrane. MCA, therefore, increases membrane tension. During T-cell activation via TCR signaling, pERM levels rapidly decrease, allowing for a relaxation of the MCA in T cells. This in turn facilitates attachment to DCs and may partially compensate for LFA-1-ICAM-1 adhesion during early interactions.[161] In support of this, regulation of stiffness is one of the factors that controls T cell engagement with APC.[162] Third, cortical tension describes the tension of the actin cortex below the plasma membrane, which depends on myosin activity, the length of actin filaments and their nanoarchitecture.[163] Together, these factors regulate the deformability of cells and the force needed to induce cell shape change. Compared to most stromal cells, leukocytes are soft and deformable cells. The Young's modulus quantifies the resistance of an object to being deformed when a force is applied to it and ranges from more than 10 kPa in fibroblasts to approximately 0.1 kPa in CD8^{+} T cells.[164] Regulation of cell deformability is tightly regulated and is likely a key feature for CD8^{+} T-cell infiltration and scanning of diverse tissues. Nonetheless, there is to date scarce information to date how the regulation of the F-actin cytoskeleton influences the biophysical properties and surveillance ability of distinct T-cell subsets in lymphoid and non-lymphoid tissues.

3 | PART 3. EXTRINSIC AND INTRINSIC REGULATION OF CD8^{+} T-CELL POSITIONING IN NLT

A major challenge is to translate findings from reductionist in vitro systems to physiological settings in vivo. What are the

contributions of leading-edge F-actin production, cortex contractility, chemokine signaling, and integrin adhesion to CD8^{+} T cell immune surveillance in lymphoid and non-lymphoid microenvironments? 2PM intravital imaging of CD8^{+} T cells within lymphoid and non-lymphoid organs has uncovered distinct motility parameters including speeds or directionality. SLO support the highest migratory speeds of 12-15 µm/min, whereas in dermis and other connective tissues speeds decrease to approximately 5-8 µm/min. In the epidermis, speeds drop to less than 2 µm/min, with the cell shape changing from ameboid in LN and connective tissue to convoluted, dendrite-rich shapes in the epidermal layer. Although it has not yet been formally shown, the shape changes and speeds displayed by CD8^{+} T cells are likely imprinted by architectural constraints of their microenvironments, including the intercellular adhesiveness of their components by tight junctions, and the availability of paths of low resistance.[13] A second, non-exclusive option is that biochemical factors of the environment such as availability of metabolites, chemoattractants, and adhesion molecules imprint the motility behavior of CD8^{+} T cells in NLTs. Yet, it is well established that leukocytes are able to migrate in the absence of surface integrin or the integrin-cytoskeletal linker talin.[108] In cell lines, low friction within microchannels suffices to generate sufficient force for cell motility.[165] Similarly, "chimneying" between channel walls has been described as mechanism for cell translocation, in a process reminiscent of mountaineers climbing between juxtaposed rock walls.[166] An advantage of non-specific guidance is that it would permit unrestricted spread of patrolling memory CD8^{+} T cells to environments with different adhesive landscapes.

In NLT, there is strong evidence that chemoattractants are important factors governing CD8^{+} T$_{EFF}$ accumulation at inflammation hotspots. As outlined above, CXCR3 ligands have emerged as attractants for CD8^{+} T cells to viral foci during skin reinfection or toward tumors.[71,72,167] Similarly, epidermal T$_{RM}$ require Gαi signaling for their dendrite shape and motility.[168] Furthermore, NLT chemoattractants play a critical role to recruit cells across tissue barriers, in particular BM oriented perpendicular to the migration path. BM consists of collagen IV, laminins, and the cross-linking proteins nidogen and perlecans.[169,170] For diapedesis in postcapillary venules, it has already been shown that neutrophils prefer areas with low density of ECM deposition for transmigration.[171] Along the same line, the BM components laminins α4 and α5 differentially affect the transmigration capacity of effector T cells.[172,173] Similarly, the epidermis with the tightly packed layers of keratinocytes as example of an epithelial tissue is separated from the underlying dermis by a dense BM. Thus, KLRG-1^{low} effector CD8^{+} T cells entering dermal vessels during skin inflammation move toward the epidermis only when attracted by CXCR3 ligands and other chemoattractants.[75] Chemoattraction is necessary because the leukocytes would otherwise not cross the BM separating dermis from epidermis owing to the dynamic properties of the cortical actin network to move away from obstacles. Thus, local chemokine receptor activation at the interface with the BM and the T-cell membrane overrides the intrinsic property of F-actin networks to induce a turn (Figure 2).

Yet, in vitro activated T cells are able to show motility within collagen matrices in the absence of chemokine addition.[117] It is therefore conceivable that NLT CD8^{+} T cells intrinsically regulate the threshold for actomyosin-driven polarization, F-actin treadmilling and contractility in a given microenvironment for spontaneous motility independent of extrinsic factors. In fact, there is evidence that CD8^{+} T cells do so as part of the surveillance strategy of their target organs. Thus, naive CD8^{+} T cells move on the stromal backbone of SLO by CCL21-triggered F-actin polymerization at the leading edge,[132,138] whereas uropod contractility plays only a minor role for lymphoid tissue scanning.[27] This is compatible with the lack of immotile obstacles in this organ—although it is tightly packed with lymphocytes, all cells remain highly mobile and move on a loose sponge-like scaffold. In contrast, T$_{RM}$ migrating in lung or epidermis become increasingly susceptible to inhibition of uropod contractility, either owing to tissue constraints or because contractility becomes increased in these cells.[168,174] In support of this, we have observed that T$_{RM}$ populations isolated from exocrine glands display high steady-state pMLC levels, indicative of a hypercontractile phenotype. This correlated with confinement-induced spontaneous T$_{RM}$ motility in the absence of chemoattractant signaling or specific adhesion (NR and JVS, unpublished observations). Recent reports have confirmed that low adhesiveness under mild confinement induces spontaneous ameboid motility via cortical contractility, even in adherent mesenchymal cell lines[175,176] (Figure 3). Such a migration mode permits to bypass the necessity for continuous expression of inflammatory chemokines after clearance of infection, which may otherwise lead to continuous leukocyte influx and exacerbated inflammation. Instead, this mode would allow CD8^{+} T$_{RM}$ to remain responsive to inflammatory chemokines that are locally secreted at sites of pathogen re-emergence. Yet, to which extent NLT CD8^{+} T cells engage in an intrinsically driven, chemoattractant- and adhesion-free motility mode that adapts to the topography of the microenvironment has not been comprehensively explored in vivo.

4 | CONCLUDING REMARKS

CD8^{+} T cells have evolved to scan the surfaces of other cells in virtually any tissue of vertebrates, which makes these cells a useful tool to study adaptation to distinct microenvironments. In recent years, many new and exciting concepts were proposed in the field of cell migration using sophisticated in vitro systems. A major challenge is to translate these findings to the physiological environments where T cells naturally move. While chemoattractants and adhesion molecules play central roles for T-cell recruitment from blood and toward activated cell clusters, basic biophysical properties of the F-actin network, membrane properties such as MCA and local contractility may favor spontaneous motility under certain circumstances. Thus, one option is that throughout their life span from naive to effector and memory CD8^{+} T cell, these

cells dynamically regulate the intrinsic threshold for dynamic F-actomyosin network remodeling. This could be achieved either by increasing F-actin treadmilling, regulating levels of actin network cross-linkers, or by adapting local or global membrane tension including Myosin II-mediated contractility. In such a scenario, chemokines may act less to trigger scanning per se but rather to direct this migration to a target area.

The mechanosensing actomyosin network of moving CD8^{+} T cells is tuned to sense the local topography to explore possible migration paths, in particular with regard to the size restriction imposed by the nucleus. A fertile field for future research is, therefore, the regulation of the nuclear-cytoskeletal linkage in CD8^{+} T-cell subsets during adaptive immune responses.[177] Yet, a confounding factor for in vivo analysis is that there is still very limited information available on the biophysical properties of many adult mouse organs. A recent study has employed super-resolution shadow imaging to assess the extracellular space of brain parenchyma.[178] This promising approach may be applicable more broadly to other NLT, especially in combination with treatments to strain the tissue such as increasing osmotic stress or physical perturbations.

Finally, the applicability of adoptive T-cell therapy for cancer immunotherapy opens a window of opportunity to selectively interfere with regulators of the actomyosin cytoskeleton, at least in preclinical rodent models. How could one improve the autonomous infiltration properties of transferred CD8^{+} T cells? T cells avoid dense ECM barriers, presumably to avoid nuclear rupture.[179] This restricts their potential to infiltrate solid tumors, which are often surrounded by BM or other barriers, in the absence of a chemokine gradient. By increasing the tolerance to elastic deformation, even at the expense of nuclear integrity, CD8^{+} T cells may be designed to become autonomous infiltrators across BM surrounding solid tumors. While this induces a non-physiological behavior of T cells, cellular integrity is less important in transferred cell populations since cotransferred, untreated cytotoxic T cells may profit from the breach-inducing activity of "infiltrators." Yet, such translational aspects of modulation of cell motility still require experimental exploration. In sum, there is still a lot to explore on how CD8^{+} T cells integrate external and internal regulators to "search" in order to "destroy."

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors report no financial or personal conflicts of interest.

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