

REVIEW ARTICLE

# Actin cytoskeleton control of the comings and goings of T lymphocytes

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## Abstract

T lymphocytes are key players of adaptive immune responses. Upon recognition of specific peptides presented by human leukocyte antigen (HLA) molecules on antigen presenting cells (APC), these cells execute subset-related functions such as killing, help and regulation. The ontogeny, the activation and the effector functions of T lymphocytes are all steps of T-lymphocyte life cycle that rely on high motility properties. These cells travel through the organism in a succession of steps, including entry into tissues, interstitial migration, APC scanning, synapse formation and tissue exit. Such ability is possible because of a plastic motility behavior, which is highly controlled in time and space. The molecular basis for the adaptable motility behavior of T lymphocytes is only starting to be unraveled. The scope of this review is to discuss recent data pointing to the key role of regulators of actin cytoskeleton remodeling in tuning distinct aspects of T-lymphocyte motility during their entry, residency and exit from tissues.

## Introduction

Fighting infections is a matter of mobilizing defense mechanisms quickly and to the right spot. As key components of the adaptive immune system, T lymphocytes orchestrate antigen-specific responses that ultimately result in the eradication of infectious agents. The mechanisms by which T lymphocytes mature, get activated and exert their function are intimately bound to their high motility behavior (1). Indeed, T-lymphocyte life cycle is linked to the transient passage into different organs, including the thymus during ontogeny and maturation, secondary lymphoid organs (SLO) during antigen-specific activation and peripheral tissues during execution of their function. A fascinating aspect of T-lymphocyte biology is the ability of these cells to regulate motility. On one hand, T lymphocytes travel at high speed over long distances as they survey most locations in the organism, in search for cellular partners presenting antigens to which they are specific. On the other hand, T lymphocytes halt migration and establish prolonged contacts with partner

or target cells as they recognize specific antigens. This ability to regulate motility is particularly evident in the context of antigen presenting cell (APC) scanning. T lymphocytes face the dilemma of losing no time in finding the right APC and taking the time to respond to antigen stimulation. Also fascinating is the ability of T lymphocytes to respond to multiple motility cues and to migrate through many various environments of distinct extracellular matrix (ECM) composition, cellular build-up, compactness and stiffness.

Which type of molecular framework controls the highly tuned plasticity of T-lymphocyte motility? Since long, the actin cytoskeleton, as a dynamic regulator of cell shape, has been known to play a key role in driving T-cell motility. Thanks to recent advances, in particular in live cell imaging, we start to understand the precise role of some of the numerous molecules coordinating actin cytoskeleton remodeling. Actin cytoskeleton can be seen as the molecular platform that senses and integrates signals of both chemical and physical nature into force-generating structures to control motility. This review will illustrate this area of research by presenting recent findings and by showing how actin regulators are involved in complex and highly tuned mechanisms of T-cell motility control.

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## Actin cytoskeleton regulation in T lymphocytes

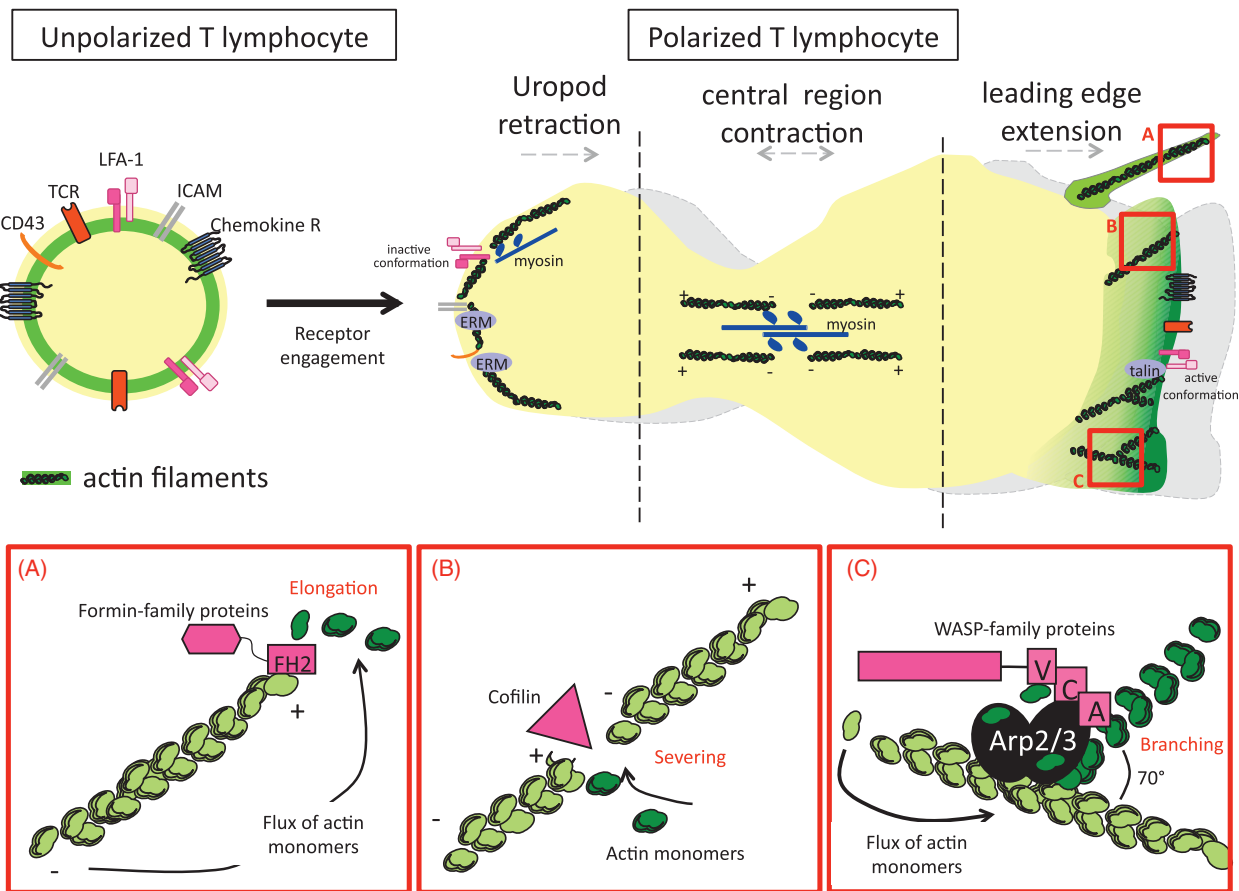
### T-lymphocyte motility: parameters and shape

T lymphocytes continuously travel between several specialized sites of the body where they acquire and exert their effector functions. After maturing in the thymus, naive T lymphocytes gain the blood circulation and enter into SLO by crossing specialized blood vessels called high endothelial venules (HEV). Once inside lymph nodes, T lymphocytes are extremely motile, a behavior permitting them to make successive contacts with several APC. Upon specific antigen recognition, T cells stop to migrate, get activated, proliferate and subsequently leave lymph nodes by extravasation. During this process, they pass through the efferent lymphatic vessel and regain the blood circulation. There, circulating activated T cells interact with activated endothelial cells and, ultimately, transmigrate into effector sites of immune response. Over the past decade, intravital two-photon microscopy has been a technological breakthrough in allowing the direct observation of T lymphocytes crawling within live tissues, including thymus, SLO and peripheral tissues (2–4). This fluorescent imaging technology, with a high penetration power, allowed not only to characterize T-cell motility but also to highlight their interaction with the environment and cell partners. Nowadays, it is established that T lymphocytes are among the quickest cells as they crawl through tissues, reaching speeds up to 25  $\mu\text{m}/\text{min}$ , and displaying an average velocity of approximately 10  $\mu\text{m}/\text{min}$  (5). T lymphocytes adopt a motility strategy known as amoeboid migration (reminiscent of *Amoeba proteus* motility), consisting in a 2-min periodic morphological change, switching from round to elongated shape. A migrating T lymphocyte harbors an actin-rich leading edge, a contractile central region and an adhesive uropod (Figure 1). It is the sequence of the following three steps that governs amoeboid migration: leading edge extension, central region traction and uropod retraction (6). The extraordinary morphological plasticity of T lymphocytes allows them to rapidly crawl through dense tissues in a manner not requiring matrix degradation, as shown in three-dimensional (3D) collagenous matrices (7, 8). Actually, T cells make use of their tissue environment to move. Indeed, they migrate along the fibroblastic reticular cell network as substratum for their motility within lymph nodes (9). As T lymphocytes travel in environments harboring different physical, cellular and molecular build-ups, it is conceivable that they adapt their amoeboid migration to their environment. In accordance with this view, *in vitro* experiments have shown that T lymphocytes adopt a myosin-dependent ‘walking’ mode during migration in a low-adhesive 3D environment, while they adopt an actin-dependent ‘sliding’ mode during migration on highly adhesive 2D surfaces (10). In addition to the adhesive properties of the environment, T lymphocytes perceive chemokines as diffusible molecules to selectively recruit cells to specific tissue locations. Depending on their maturation stage and their functional subset, T

lymphocytes express different sets of chemokine receptors that will drive tissue homing, positioning in precise tissue areas, as well as tissue exit (11). Finally, T cell encounter with partner cells will deliver further motility signals. While noncognate APC are perceived as a trigger of homeostatic motility (12), the encounter with APC presenting cognate peptide-major histocompatibility complex (MHC) complexes will induce a T-cell receptor (TCR)-driven motility stop signal (13).

### Actin cytoskeleton as a key component for polarization and force generation

How are the motility signals integrated by T cells and converted into an appropriate migratory behavior? The onset of directional migration requires cell polarization and force generation. Both mechanistic events rely on actin cytoskeleton dynamics. T-cell polarization corresponds to actin cytoskeleton filament reorganization, switching from a radial distribution in unpolarized T cells to a linear symmetric organization in polarized T cells (Figure 1). Actin cytoskeleton involvement in T-cell polarity was shown by the use of drugs such as cytochalasin D and latrunculin B, known to disrupt actin filaments (14). T-cell polarization arises as consequence of regulated actin cytoskeleton rearrangement in response to chemokine receptors, LFA-1 or TCR engagement (13, 15). The small GTPases of the Rho family act as key integrators of stimuli received by membrane receptors by selectively activating actin regulators. The identity of Rho GTPases and their molecular regulation in T cells have been reviewed elsewhere (16, 17) and will not be covered in this review. At the leading edge of a polarized T lymphocyte, a 0.1–0.2  $\mu\text{m}$ -thick lamellipodium is formed. It contains short and branched actin filaments that are continuously produced at proximity to the plasma membrane and degraded at the rear of the lamellipodium, as shown 20 years ago by fluorescent microscopy (18). Released actin monomers are recycled at the front of the lamellipodium, producing a retrograde actin flux. This flux generates a traction force that permits cell forward extension and crossing through small gaps of the extracellular matrix. It has been proposed that the generated traction force is not due to actin filament elongation but instead depends on the conversion of released thermal energy in elastic energy (19). Moreover, the enrichment of chemokine receptors (CCR2, CCR5 and CXCR4) at the leading edge of T lymphocytes allows them to sense the environment and to direct their migration (20). The integrin LFA-1 was also shown to be enriched at the leading edge (21). Its interaction with its ligand intercellular adhesion molecule-1 (ICAM-1) initiates lamellipodium formation. In concert with talin, vinculin, focal adhesion kinases and  $\alpha$ -actinin, LFA-1 connects actin cytoskeleton to the substratum, leading to the formation of new adhesive points (22). Behind the leading edge, the central region brings the traction force required for T-cell contraction. Myosin association to actin filaments and sliding toward the filaments



**Figure 1** T-lymphocyte motility is sustained by polarization and actin cytoskeleton remodeling. Unpolarized T lymphocyte harboring a radial distribution of actin filaments. Upon chemokine receptor, T-cell receptor (TCR) or LFA-1 engagement, the T lymphocyte polarizes and forms a protrusive leading edge, a contractile central region and an adhesive uropod required for amoeboid locomotion. In polarized T lymphocytes, actin cytoskeleton distribution follows a linear symmetry and membrane receptors are enriched at the leading edge and/or at the uropod. The A, B and C zooms show three different modes of actin cytoskeleton nucleation. (A) Actin filament elongation promoted by the formin protein family. (B) Actin filament severing by cofilin. (C) Actin filament branching, with a 70° angle, induced by proteins of the Wiskott–Aldrich syndrome protein (WASP) family together with the Arp2/3 complex. Nascent filaments and pre-existing filaments appear in dark green and light green, respectively. FH2, formin homology 2 domain; VCA, Verprolin and Cofilin homology domain, Acidic domain; (+) barbed end; (–) pointed end.

pointed ends induce their contraction in an ATP-dependent manner. The uropod function in amoeboid migration is not yet completely understood. It was shown that the uropod cycles continuously between substratum adhesion, retraction and substratum deadhesion, favoring T-lymphocyte migration. Moreover, the adhesive molecules CD43, CD44 and ICAM-1, 2 and 3 are enriched at the uropod, certainly due to their association with ezrin, radixin, moesin (ERM) family protein, linking them to actin cytoskeleton (23). At the uropod, myosin permits LFA-1/ICAM-1 dissociation and consequently its deadhesion to the substratum (15).

**Protein families controlling actin cytoskeleton remodeling**

Actin cytoskeleton remodeling is in part based on an assembly/de-assembly cycle, whereby actin filaments are built

at one end by assembly of actin monomers (actin polymerization or nucleation), while actin filaments are de-assembled (actin depolymerization) at the other end (Figure 1, zoomed part). An actin filament is composed of a dynamic barbed end and a less active pointed end. This kinetic difference permits filament elongation at one end and shortening at the other end. Actin filaments can be assembled and remodeled in different ways by distinct families of actin-associated molecules, including actin nucleators, actin bundling proteins and actin capping proteins (Table 1). Members of the ERM family control cell rigidity by linking cell surface proteins to the cortical actin cytoskeleton. Although they play an important role in T-cell motility (24), their function will not be detailed here as they do not remodel actin filaments *per se*. Actin nucleators studied so far in T lymphocytes comprise the formin family, cofilin and the Arp2/3 complex (Table 1). Formin family

**Table 1** Actin cytoskeleton regulators identified in T lymphocytes

| Type of actin remodeling | Protein family        | Protein name               | Mode of action                  | References           |
|--------------------------|-----------------------|----------------------------|---------------------------------|----------------------|
| Actin nucleation         | Formins               | mDIA1-3                    | Elongate actin filaments        | (37)                 |
|                          |                       | FMNL1                      |                                 | (83)                 |
|                          | Cofilin               | Cofilin                    | Severing actin filaments        | (84)                 |
|                          |                       | Arp2/3-associated proteins |                                 | Positive regulators: |
|                          | WASP                  |                            | (45, 85)                        |                      |
|                          | N-WASP                |                            | (34)                            |                      |
|                          | WASH                  |                            | (86)                            |                      |
|                          | WAVE2                 |                            | (49)                            |                      |
|                          | HS1                   |                            | (35, 36)                        |                      |
|                          |                       | Negative regulators:       |                                 |                      |
|                          | Coronin-1A            |                            | (61, 62)                        |                      |
| Actin bundling           | Plastin               | L-plastin                  | Bundle parallel actin filaments | (87)                 |
| Actin capping            | Actin capping protein | Gelsolin                   | Stabilize actin filament length | (88)                 |

WASP, Wiskott–Aldrich syndrome protein.

proteins (mDIA1-3 and FMNL1) nucleate and elongate long linear actin filaments, generating an unbranched actin network resembling actin cables (Figure 1A). Formins express a conserved formin homology 2 (FH2) domain that interacts with the barbed end of actin filaments. Cofilin is severing actin at the pointed end to provide free barbed ends for actin polymerization and depolymerization (Figure 1B). The Arp2/3 complex branches new actin filaments to pre-existing filaments with an angle of 70° (Figure 1C). Its activation is driven by two protein families: the Wiskott–Aldrich syndrome protein (WASP) family including WASP, neural Wiskott–Aldrich syndrome protein (N-WASP), WASH and the WAVE family including WAVE1, 2 and 3. These proteins contain a composite VCA domain (Verprolin and Cofilin homology domain, Acidic domain) at their C-terminal extremity. The V domain links an actin monomer whereas the C and A domains interact with the Arp2/3 complex, leading to the branching of new actin filaments. WASP is the best-studied actin regulator in hematopoietic cells, including T lymphocytes, because its deficiency is the cause of the Wiskott–Aldrich syndrome (WAS), a rare primary immunodeficiency. Although WAS gene mutations resulting in the absence of WASP expression are associated with severe clinical manifestations (high susceptibility to infections, autoimmunity and tumors), WASP shall not be considered the only actin regulator of hematopoietic cells. Actually, WASP deficiency results only in mild defects of T-cell motility. If one considers that at least part of the defects in T-cell activation and effector functions in WAS are due to T-cell motility alterations, one appreciates how a fine-control of actin-dependent motility is a key element in the tuning of T-cell responses.

HS1, the hematopoietic homolog of cortactin, which functions in stabilizing branched actin filaments, is also an Arp2/3 complex promoting factor expressed in T cells. However, its role in T-cell polarity and motility has not yet been examined. Coronin-1A, belonging to the coronin family protein, which associates with and inhibits the Arp2/3 complex, is expressed

in T cells as well. It associates with and inhibits the Arp2/3 complex. Recently, the case of one patient presenting clinical manifestations of primary immunodeficiency was linked to coronin-1A deficiency. This is in addition to WAS, an example of severe clinical disorders related to the absence of one actin cytoskeleton remodeling protein. In addition to nucleation, actin filaments can be remodeled by actin bundling proteins. The latter assemble several actin filaments together following the same axis. T cells express the actin bundling protein L-plastin, which was shown to be involved in T-cell polarity and migration. Finally, actin capping proteins, like gelsolin, stabilize length of actin filament by preventing actin monomer association as well as dissociation at the filament barbed end. Over-expression of gelsolin in T lymphocytes inhibits their spreading on plate-bound CD3 antibodies. However, gelsolin involvement in T-cell polarization and motility still needs to be investigated.

## Actin-based control of T-lymphocyte differentiation and activation

### Intrathymic T-lymphocyte genesis

From development to final effector function, the high motility of T cells clearly serves critical functions of adaptive immunity. Such a motile behavior is already present at all steps of thymopoiesis, from precursor entry until mature thymocyte egress (25). Maturation of  $\alpha\beta$  T cells is initiated as thymic progenitor cells move into the thymus through blood vessels located at the cortico-medullary region. Notably, the complex developmental program of T cells is intertwined with several migratory steps (25). The most immature thymocytes, defined as CD4<sup>-</sup>CD8<sup>-</sup> double-negative (DN), first move outward to the subcapsular region of the cortex. After TCR $\beta$  gene rearrangements and intense proliferation, they become CD4<sup>+</sup>CD8<sup>+</sup> double-positive (DP) cells (26). Following positive selection, the DP thymocytes then display a

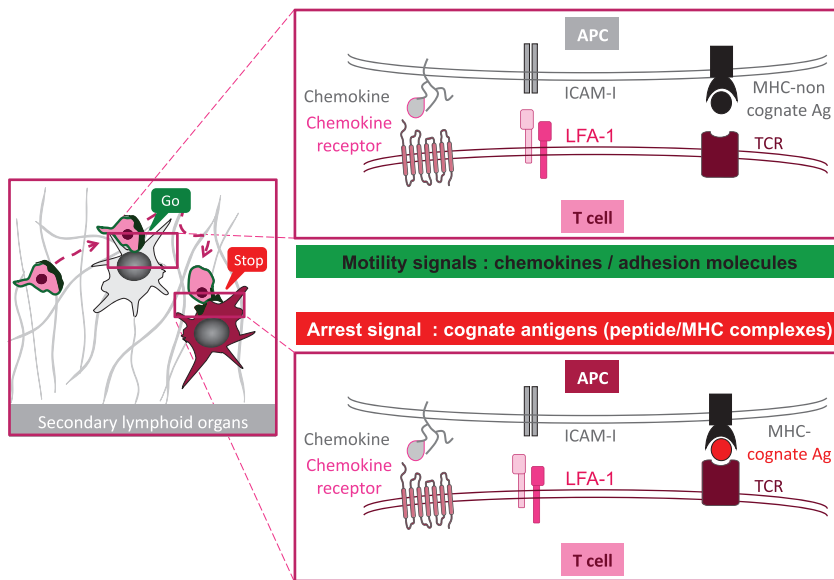
very directed motility back to the cortico-medullary region that they need to cross to enter the chemokine-rich medulla (27). In this location, thymocytes that differentiated to CD4 or CD8 single-positive (SP) subsets and bearing TCR that strongly recognizes self-antigens will be eliminated through negative selection. The relatively few surviving cells become mature thymocytes and gain blood circulation through the perivascular space. Their egress is regulated *via* sphingosine-1-phosphate receptor 1 (S1P1), which allows sensing of high blood concentration of S1P (28) and *via* CXCR4, which on the opposite mediates chemorepulsion to high CXCL12 thymic concentration (29).

Adhesive and migratory steps of developing thymocytes define them as highly motile cells, as indicated by several microscopic approaches (30, 31). Their journey through distinct thymic microenvironments is regulated by chemokine gradients and extracellular matrix molecules (32, 33). In this context, several molecular controls based on actin regulation of T-cell motility have clear impact on  $\alpha\beta$  T-cell maturation (Figure 3). The WASP family members WASP and N-WASP, Cdc42 effector proteins involved in Arp2/3-mediated actin polymerization, were shown to have a redundant function in T-cell development (34). Despite the indispensable role of WASP for *in vitro* TCR-induced proliferation and actin-dependent spreading response (an assay to model the contact area between T cells and APC) in thymocytes, either WASP- or N-WASP-deficient mice seem to carry out normal T-cell maturation. However, conditional gene targeting of N-WASP in WASP-KO thymocytes leads to aberrant T-cell maturation, as shown by thymic hypocellularity, lower DN cell cycling and relative retention of SP cells, concomitantly to more pronounced defects in migration and spreading (34). Deficiency in HS1 shows no major defects in lymphocyte development, but impaired negative selection was reported (35, 36). Mouse genetically deficient in the formin family member mDia1, which is activated by the RhoA small GTPase and function in Arp2/3-independent unbranched nucleation of actin filaments, have reduced thymocyte chemotaxis, increased retention of the most mature SP cells and lower number of T cells in the periphery (37). Another molecule involved in transduction of chemokine response, the actin bundling protein L-plastin (38), was shown to affect the signaling machinery of actin polymerization in thymocytes. Interestingly, a subtle regulation of cell motility by L-plastin seems to function in thymocytes. Although required for chemokine-driven migration and thymocyte egress, this molecule is dispensable for proximal CCR7 signaling, as early Rac activation and actin polymerization occur normally in L-plastin deficient thymocytes following CCL19 stimulation (38).

By using two-photon microscopy and mouse gene targeting, we begin to understand the identity of signals controlling trafficking of T cells through their intrathymic developmental journey. However, fine actin-based regulation of thymocyte motility is still poorly explored.

### T-lymphocyte activation in SLO: search, stop and go

Naive circulating T lymphocytes gain SLO by crossing HEV. This finely controlled thoroughway is initiated by a CD62L dependent rolling of T cells on HEV, permitting them to sense the chemokines CCL19 and CCL21 presented at the vessel surface. CCR7 activation induces LFA-1 conformational change leading to firm adhesion of T cells on HEV and to their diapedesis (39). Once inside SLO, T cells explore the environment in search of APC bearing cognate antigens (Figure 2). This task requires that T cells scan carefully APC surfaces and, at the same time, avoid potential time-consuming adhesion in non specific conjugates. Naive T-cell activation in SLO occurs in three distinct motility phases (3). First, naive T lymphocytes establish successive short contacts preferentially with dendritic cell (DC) dendrites. Serial gathering of stimuli diminish the threshold for successive activation (40). In a second phase, T-lymphocyte mean velocity diminishes considerably permitting long lasting contact with DC. Third, T cells dissociate from DC and regain higher velocity. The recognition of specific peptide-MHC complex on APC is the critical event for T cell-mediated adaptive immune response. One major consequence of TCR activation is the actin cytoskeleton rearrangement that contributes to the formation of an immunological synapse at the T cell/APC interface (41). The dynamic signaling platforms that are assembled at the immunological synapse are also under influence of the underlying actin cytoskeleton (42), indicating that actin cytoskeleton remodeling controls multiple steps of T-cell activation (Figure 3). Among the main regulatory proteins are the small GTPases Cdc42 and Rac and their effector molecules WASP and WAVE2, respectively. These molecules are present at the immunological synapse and at the leading edge of lamellipodia, where they regulate actin reorganization by direct interaction with the actin nucleator Arp2/3 complex (43). WASP deficiency results in aberrant T-cell activation (44, 45). WASP-driven actin nucleation appears to be dispensable for T cell/APC conjugate formation and firm adhesion (46). Initial studies have concluded on different requirements for WASP in immunological synapse assembly (44, 47–49). However, recent approaches have shown that WASP stabilizes the synapse formed during both cognate and noncognate encounters (50–52). Deficiency in WAVE2 impairs T-cell activation and adhesion to APC and to fibronectin (43). WAVE2 may act prior to WASP in promoting actin-rich membrane protrusions during adhesion to the APC and initiation of synapse formation (49). As a result, its deficiency affects T cell/APC conjugate formation. T cells from mDia1-deficient mice have reduced chemotactic responses and aberrant trafficking to SLO (37). HS1 as well as cofilin, an inducer of actin severing and depolymerization, are also regarded as essential regulators of T cell actin dynamics, because either HS1-deficient T cells or blocking of cofilin binding to actin impairs immunological synapse organization, proliferation and cytokine production (36, 43). Therefore, by decoding various stimulatory signals into actin cytoskeleton



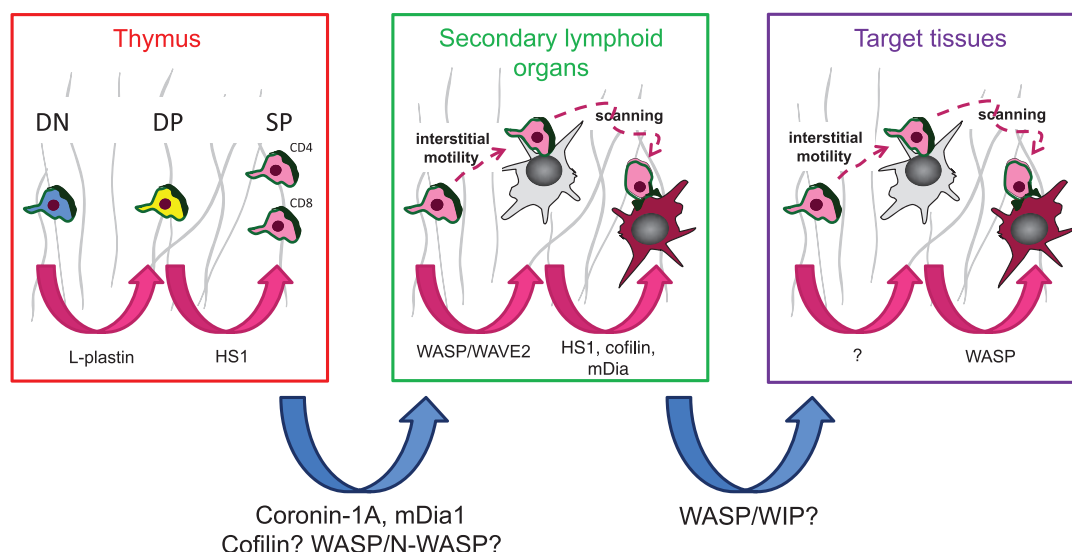
**Figure 2** T-lymphocyte motility and arrest on antigen presenting cells in secondary lymphoid organs (SLO). In SLO, T lymphocytes will successively encounter noncognate and cognate antigen bearing antigen presenting cell (APC). In response to chemokines and adhesive molecules present at the surface of noncognate APC, T lymphocytes receive motility signals (upper panel). In addition to these signals, the recognition with the T-cell receptor (TCR) of peptide/major histocompatibility complexes (MHCs) at the surface of cognate APC leads to a stop signal, which induces T cell arrest (lower panel).

remodeling, WASP, WAVE2 and HS1 may act in a nonredundant concerted fashion to regulate the different dynamic steps of immunological synapse assembly.

### Actin-based control of T-lymphocyte effector functions in peripheral tissues

A key mechanism controlling lymphocyte egress from SLO is sustained S1P1 expression allowing T-cell migration across endothelial barrier into efferent sinus vessels in response to high blood S1P concentrations (28). In addition, as a result of activation, newly generated effector T cells downregulate the SLO homing molecules CCR7 and CD62L and substitute them by tissue homing chemokine receptors such as CCR4 and CCR10. This provides activated T cells reaching the blood circulation with new sensing profiles enabling them in the case of CCR4 and CCR10 to respond to CCL22 and CCL27, which will direct them towards gut and skin, respectively. Within the bloodstream, activated T lymphocytes will eventually transmigrate against hemodynamic shear forces through post-capillary venules, by performing sequentially selectin-dependent rolling, integrin-dependent tight adhesion and tissue invasion. Effector T-lymphocyte homing to specific peripheral tissues appears to be at least in part driven by the acquisition of specific molecular signatures in corresponding tissue-draining SLO. For example, T cells undergoing activation in the gastrointestinal-associated lymphoid tissue (GALT) preferentially upregulate  $\alpha 4\beta 7$  integrin and CCR9, which together drive homing to the small intestinal mucosa (53). The motility behavior of effector T lymphocytes in peripheral tissues has recently started to be explored by the use of two-photon microscopy. In an ear skin graft model, infiltration of alloreactive CD8<sup>+</sup> T cells was showed to occur in two distinct steps (54). CD8<sup>+</sup> T cells were initially

confined to the dermis-epidermis junction, suggesting access to the target tissue from its outer layers (even if this tissue was fully revascularized). After 1–2 days, CD8<sup>+</sup> T cells had disseminated throughout the graft and their velocity dropped to 2  $\mu\text{m}/\text{min}$ , probably as a result of a stop signal upon recognition and elimination of target allogeneic cells. In a model of *Toxoplasma* infection of the central nervous system, presenting much lower density of target cells than the previous model, measurements of CD8<sup>+</sup> T-cell motility revealed oscillations between runs reaching speeds of 20  $\mu\text{m}/\text{min}$  and relative pauses (55). Interestingly, sensing of the chemokine CXCL10 mainly endowed CD8<sup>+</sup> T cells with increased speed. Computational modeling led to propose that CD8<sup>+</sup> T cells follow Lévy walks rather than Brownian motion to improve the search for rare infected target cells. Lévy walks have been characterized in different animal species and are characterized by a mix of long trajectories and short, random movements. In a model of epicutaneous *Herpes simplex* virus infection, differential motility patterns have been identified between CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets in the different skin layers (56). While recirculating memory CD4<sup>+</sup> T cells were highly motile with mean velocity of 6  $\mu\text{m}/\text{min}$  and mainly localized to the dermis, CD8<sup>+</sup> T cells were confined to the epidermis showed reduced velocity and did not recirculate. Such data suggest that different T-cell subsets may harbor distinct propensity to recirculate as well as distinct tissue distribution. Together, the above cited studies indicate that effector T-cell motility behavior within target peripheral tissues reflects the capacity of T cells to control their speed to scan, stop and go as previously described for naive T cells in SLO. The intrinsic run/pause oscillations characterizing T-cell motility patterns may prove key at allowing these cells to adapt their motility to their environment including chemokines, adhesion



**Figure 3** Molecular control of T lymphocyte comings and goings throughout the organism. During their life cycle, T lymphocytes migrate to and through distinct tissues. Our knowledge of the control of the numerous T lymphocyte migration steps by actin cytoskeleton regulators is still limited (name of the identified molecules below the arrows; Table 1). During development in the thymus from double-negative (DN) to double-positive (DP) to single-positive (SP), T lymphocytes follow a precise routing (symbolized by the pink arrows) as they undergo positive and negative selection. Naïve CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes then travel through the blood circulation to enter secondary lymphoid organs by transendothelial migration (blue arrows to indicate long-range travels). Their high interstitial motility allows them to scan a high number of dendritic cells to finally stop upon antigen recognition. Following activation and acquisition of an effector phenotype, T lymphocytes will eventually gain access to nonlymphoid peripheral tissues that they will scan until identification of target cells.

molecules, peptide-MHC complexes, target cell density, ECM composition and network of reticular cells.

Today, there is still a paucity of data on how effector T-cell homing and motility in peripheral tissues are controlled *via* actin cytoskeleton remodeling (Figure 3). Most of our knowledge stems from the WASP deficiency model, in which T-cell motility defects contribute to immune dysregulation (57). Homing experiments with WASP-deficient lymphocytes showed that, after intravenous injection, they preferentially accumulate in the blood and are less prone to re-enter Peyer patches, when compared with WT cells (58). Indeed, T cells deficient in WASP or WIP (a WASP-binding partner), or even double-deficient ones, present the same defect on lymph node migratory potential (59). Those homing defects were correlated with disease as, in the experimental model of colitis induced by transfer of CD45RB<sup>hi</sup> T cells to immunodeficient mice, inflammation process was delayed and less severe following transfer of WASP-deficient T cells. This was attributed not only to overall T-cell motility impairment, but also to a pronounced defect in trafficking of regulatory T cells to inflamed areas (60). To date, the role of WASP in the motility of effector T cells within tissues has not been investigated. Our recent *in vitro* study showed that WASP-silenced CD4<sup>+</sup> T lymphocytes present an intrinsic hyper-motility behavior upon noncognate APC encounter (51). This highlights the notion that WASP (and probably actin cytoskeleton regulation in general) shall not be considered simply as a facilitator of motility,

but as a regulator able to tune motility depending on the context. Our study further indicated that the failure of WASP-deficient T cells to control APC-driven motility resulted in a reduced stop signal at low antigen concentration. It can be proposed that alteration in the tuning of WASP-deficient T-cell motility will ultimately contribute to the onset of combinatorial events of immunodeficiency and autoimmunity.

Coronin-1A deficiency was first described by a mouse phenotype of pronounced peripheral T-cell lymphopenia (Ptd mice), presenting a defect on thymic egress (61). Thus, coronin-1A is directly associated with a mouse and human severe immunodeficiency (62). Molecularly, coronin-1A plays a critical role in chemokine-regulated uropod formation, talin polarization and migration in response to CCL19. This cell motility impact seems to be independent of T-cell activation and expansion after TCR triggering, pointing to a specific actin cytoskeleton regulator primarily involved in lymphocyte trafficking. The T-cell trafficking defects associated to coronin-1A-deficiency has been related to a tolerant phenotype after skin allograft transplants (63). Additionally, the identification of *coronin-1a* gene mutation in the lupus-related Lbm3 locus clearly indicates that this gene controls the fate of pathogenic T cells. Indeed, *coronin-1a* gene mutation not only conferred suppression of lupus phenotype in mouse models but also induced tolerance when coronin-1A-deficient T cells were transferred in nondeficient *lpr* mice (64). This illustrates how an actin cytoskeleton

regulator is associated not only to severe and profound immunodeficiency but also to immune dysregulation.

The importance of a regulated control of T-cell trafficking is further illustrated by cases in which viruses subvert T-cell motility. Recently, human immunodeficiency virus (HIV) negative factor (Nef) was proposed as a protein used by this virus to hijack actin regulators and thereby modify T-cell migratory abilities (65). Initially, Nef was shown to associate with and activate PAK2, and inhibit directly the actin severin factor cofilin, causing actin turnover reduction (66). Subsequently, T-cell migration *in vitro* through transwell and transendothelial barriers was inhibited by Nef expression (67). Moreover, an *in vivo* study clearly showed that recent thymic emigrants are not able to properly perform lymph node transendothelial migration *in vivo* (65). Those results together not only reveal one potential HIV additional immune escape mechanism but also suggest cofilin as one strong candidate to induce peripheral tolerance by T cell restrictive trafficking. Recently, the intravital visualization of HIV spreading in lymph nodes of mice carrying human T cells showed reduced motility of infected CD4<sup>+</sup> T cells which was concomitant with the formation of HIV envelope-dependent multinucleated syncytia through membrane tethering and cell elongation. This ability of HIV to manipulate T-cell motility and morphology is probably a strategy to optimize cell-to-cell dissemination while still permitting trafficking to distant tissues (68). On the other hand, recruitment of virus-infected T lymphocytes into the central nervous system during human T-lymphotropic virus-1 (HTLV-1) infection is an important step in the development of virus-associated neuroinflammatory diseases. Recently, HTLV-1 infection was demonstrated to increase T-cell motility *in vitro* in a mechanism dependent on collapsin response mediator protein 2 (CRMP2) (69). This protein is a phosphoprotein involved in cytoskeleton rearrangement, previously demonstrated to have an important role in T-cell polarization and migration (70), and to induce state of HTLV-1 invasiveness and brain targeting (69).

In conclusion, further *in vivo* studies, addressed by two-photon microscopy and using computational analyses, are necessary to dissect intracellular pathways governing actin–cytoskeleton remodeling necessary to fine regulate immunosurveillance without tolerance breakup.

### Manipulation of T-cell motility in clinical settings

Although actin cytoskeleton remodeling is fundamental to the different facets of T-lymphocyte motility, further research is still needed to identify pathways and drugs that may specifically affect T-cell trafficking. To date, the most advanced approaches aiming at controlling T-cell motility target either adhesion molecules and chemokine receptors, or the stop signal. Here we provide a selection of studies on such approaches, although they do not directly target actin

cytoskeleton remodeling. This is followed by an outlook on the possibility for new cures that the in-depth knowledge of the actin-based control of T-cell motility may offer.

Given the fact that organ-specific homing of T cells is driven by a molecular signature made of a set of adhesion molecules and chemokine receptors, therapeutic strategies targeting adhesion receptors and chemokine receptors have been designed for organ-specific inhibition of chronic inflammation (71). The very first successful initiative to use motility control as therapeutic target came from the modulator of S1P receptors, FTY720 (fingolimod). This drug is currently used in clinics to prevent episodes of symptoms and slow the worsening of disability in patients with relapsing forms of multiple sclerosis (72). Another biological target of choice is the  $\alpha$ -4-integrin, which can be blocked by the humanized antibody natalizumab. This targeting was demonstrated to be efficacious for the treatment of relapsing-remitting multiple sclerosis (73). It also increased rates of remission and maintained a symptom-free status in patients with Crohn's disease (74). Ongoing clinical trials are testing the safety and efficacy of additional drugs targeting leukocyte motility, such as vedolizumab, a humanized monoclonal antibody directed against the  $\alpha$ 4 $\beta$ 7 integrin, which is currently in phase III clinical trial for the treatment of ulcerative colitis and Crohn's disease (75). For what concerns drugs targeting chemokine receptors, one may cite the example of maraviroc, a CCR5 negative allosteric modulator. Initially developed as a drug to block HIV entry, it was shown to efficiently control visceral graft-*vs*-host disease in the context of allogeneic bone marrow transplantation (76).

The T-cell stop signal is key in setting the quality of the interaction of T cells with APC (13). As such, it might be a potential target in abnormal T-cell responses. This view is supported by the finding that T cell/APC contact duration determines the development of T-cell tolerance or activation (77). In this sense, cytotoxic T-lymphocyte antigen-4 (CTLA-4) has been identified as a key regulator of the T-cell stop signal (78), and seems to be able to modulate T-cell motility in the context of anti-tumor immunity (79). Along with PD-1, another modulator of the T-cell stop signal and tolerance (80), CTLA-4 has been exploited to prevent and treat autoimmunity, promote transplant acceptance and limit tumor growth.

As shown in this review, the actin cytoskeleton is fundamental in the regulation of T-cell motility. It is therefore a potentially attractive therapeutic target to either boost or dampen T-cell responses, depending on the pathological setting. However, existing actin-targeting drugs are poorly specific and highly toxic to the heart, which prevent their clinical application. There is a current line of research aiming at impairing actin cytoskeleton remodeling in tumors, which were shown to hijack actin regulators and therefore favoring motility and invasion (81). By analogy, it is reasonable to think that pathogenic T cells, in the context of autoimmune and inflammatory disorders might have an altered expression



of specific actin regulators. Global signature studies should be helpful at identifying potential target molecules. In parallel, the search for selective inhibitors of actin cytoskeleton remodeling (82) is expected to increase the panel of drugs that can be tested in isolated T lymphocytes or relevant animal models.

### Concluding remarks

Over the past 10 years, the optimization of intravital imaging has permitted tremendous progress in our understanding of how T-lymphocyte motility behavior regulates the different cell developmental stages. Still, much remains to be done to gather a complete picture of motility behaviors of the different T-cell subsets in particular tissue compartments. So far, we have reached only a partial view on how T lymphocytes integrate information collected along their paths into adapted motility programs. As reviewed here, our knowledge of the molecular regulation of actin dynamics is limited to a handful of molecules that have been studied individually. With further development of intravital imaging and availability of new knockout murine models, the list of actin regulators contributing to T-cell motility will grow. It will be interesting to also design *in vitro* studies aiming at unraveling how different actin regulators combine their function to tune actin cytoskeleton dynamics during T-cell chemotaxis and assembly of immunological synapses. An additional challenge will be to address whether the modulation of the activity of specific actin regulators can be exploited for therapeutic strategies to tune T-cell motility and functional performance.

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### Conflict of Interest

The authors have declared no conflicting interests.

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