

# LFA-1 in T cell priming, differentiation, and effector functions

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## Abstract

The integrin LFA-1 is crucial for T cell entry into mammalian lymph nodes and tissues, and for promoting interactions with antigen-presenting cells. However, it is increasingly evident that LFA-1 plays additional key roles beyond the mere support of adhesion between T cells, the endothelium, and/or antigen-presenting cells. These include roles in homotypic T cell:T cell communication, the induction of intracellular complement activity underlying Th1 effector cell polarization, and the support of long-lasting T cell memory. Here, we summarize briefly current knowledge of LFA-1 biology, discuss novel cytoskeletal regulators of LFA-1 functions, and review new aspects of LFA-1 mechanobiology that are relevant to its function in immunological synapses and in specific pathologies arising from LFA-1 dysregulation.

## LFA-1: beyond cell adhesion

Lymphocytes in the blood have the ability to migrate into lymph nodes (LNs) and to peripheral sites of injury, infection and inflammation by utilizing members of two major adhesion receptor families, selectins and integrins [1]. Integrins comprise a family of over 20 heterodimers that can modulate their adhesiveness in response to inside-out signaling pathways through conformational changes and alterations in receptor clustering via reversible cytoskeletal associations [reviewed in [2]]. One of the major leukocyte integrins on T cells is **LFA-1** (CD11a/CD18 or  $\alpha$ L $\beta$ 2), which, apart from orchestrating the **antigen-presenting cell (APC)/T cell** interface in an **immunological synapse (IS)**, plays roles in T cell:T cell interactions, T cell proliferation, the induction of T cell effector functions, and T cell memory development. Other integrins on T cells include different  $\beta$ 1 integrin members such as VLA-4 ( $\alpha$ 4 $\beta$ 1) and VLA-5 ( $\alpha$ 5 $\beta$ 1), which are, however, more specialized and not as well studied as LFA-1. Here, we take the reader along the journey of a T cell through the healthy and diseased host and specifically highlight novel LFA-1 functions during this voyage that go beyond its role as a simple adhesion receptor. Furthermore, we introduce novel molecular regulators and discuss how the interplay of LFA-1 with cytoplasmic effector molecules is necessary for both its adhesive and signaling functions.

## **T cell entry into lymph nodes and T cell activation**

The entry of naïve lymphocytes into lymph nodes requires their arrest on specialized post capillary venules, high endothelial venules (HEVs) via LFA-1 interactions with two immunoglobulin superfamily ligands, **ICAM-1** and **ICAM-2** [3]. LFA-1 is maintained in inactive states on naïve T cells in blood but undergoes *in situ* modulation to generate high affinity and **avidity** for these ligands (Figure 1, Box 1). Both *in vitro* and *in vivo* data suggest that LFA-1 adhesiveness can be triggered during sub-second contacts of T cells with chemokines presented on HEVs [4-7] (Figure 2) through **inside-out signaling** pathways [8].

Following entry into lymph nodes, naïve T cells migrate rapidly and preferentially on **FRCs** that display high amounts of CCR7 chemokine ligands CCL21 and CCL19, on their surface [9] and on professional APCs, mainly dendritic cells (DCs) [10]. This is essential for their search for

cognate peptide-MHC (pMHC) complexes presented on DCs [11, 12]. T cells initially engage DCs presenting cognate antigenic MHC-peptide complexes via multiple (serial) transient, minute-lasting contacts (kinapses) [13], before they generate long-lasting contacts (IS) with these antigen-presenting DCs, which is required for full activation [14, 15] (Figure 2, and see below).

The IS is a radially symmetric contact interface with a core containing accumulated TCR, protein sorting and secretory compartments surrounded by an LFA-1/talin rich intermediate ring and a peripheral actin rich ring [16]. Those three regions were initially discovered by Kupfer and colleagues in a land mark microscopy study on T cells and B cell APCs and were termed the central Supramolecular Activation Cluster (**cSMAC**), peripheral SMAC (**pSMAC**) and distal SMAC (**dSMAC**) [17]. LFA-1 crucially contributes to the formation and function of stable ISs [18]. Like other integrin bonds LFA-1-ICAM-1 bonds are stabilized by forces, which require both molecules to be properly anchored to the cortical cytoskeleton (Box 1).

### **The three phases of T cell motility and arrest on DCs.**

Classical intravital microscopy studies in mice have introduced a unified paradigm where T cell activation occurs in three main phases of T cell motility and arrest on DCs, triggered by distinct antigenic signals and contexts of immunization [19, 20]. During the first phase, lasting 6-12 hours, naïve T cells migrate rapidly on FRC stromal elements and on their associated DCs until they encounter either resident or migratory DCs that present cognate pMHC complexes, in a process termed serial **TCR triggering**, originally observed *in vitro* [21, 22]. Naïve T cells integrate short lived TCR signals from different pMHC presenting DCs until a critical activation threshold is achieved [23], but they rarely arrest on DCs during this early phase [19, 24]. The duration of this phase depends among other factors on the strength of the integrated TCR signals, hence, on the affinity of the pMHC and their relative density [14]. The naïve T cells may use this modality of serial TCR activation for keeping their high motility and increasing their probability of encounter of rare Ag-presenting DCs. These T cells also integrate many weak signals from resident DCs that present low density pMHC complexes transferred by **cross- presentation** from singular migratory

DCs [11, 25-27]. During the second phase, which lasts 4-12 hours, T cells lose their motility and generate prolonged TCR specific IS [19]. Individual ISs can last several hours and interference with their stability may lead to tolerance or **anergy** [28]. This second phase lasts 4-12 hours and is followed by a third phase depicted by T cell detachment from the DCs, followed by extensive **swarming** of the detached T cells around a cluster of DCs, possibly governed by local chemokinetic signals or by T cell:T cell associations [12].

How LFA-1 is used by T cells during the different priming phases is not completely understood. intravital microscopy studies in skin draining lymph nodes of mice immunized with OVA targeted to skin and lymph node DCs [29], indicated that during phase I, naïve transgenic CD4<sup>+</sup> T cells could arrest on DCs in an antigen-dependent manner, but independently of DC-expressed ICAMs, giving rise to T-DC kinapses [29] (Figure 2). These data argue that at least in the first phase after their entry into the **T zone** of reactive lymph nodes, T cell apparently keep their LFA-1 in an inactive state that fails to generate firm adhesion with DC-presented ICAMs. Nevertheless, CD4<sup>+</sup> T cells can, arrest on exogenous LPS-stimulated BM derived DCs pulsed with saturating amounts of cognate antigenic peptides that enter skin draining lymph nodes through lymphatics [30]. We therefore favor the possibility that following entry into the T zone, during the first hours of T cell scanning of resident endogenous DCs within the T zone, both CD4<sup>+</sup> and CD8<sup>+</sup> T cells fail to switch their LFA-1 in response to antigenic signals. Consistent with this, in vitro LFA-1 on human T cells was shown to undergo a switch to high affinity conformation in response to chemokine signals, but not upon potent TCR activation with anti-CD3 antibodies [31].

It is unclear at what phase and where serially TCR triggered T cells switch their LFA-1 into the highly adhesive state capable of generating prolonged arrests on antigen-presenting DCs. We postulate that activated T cells (T blasts) that have acquired late TCR activation markers such as CD25 may have a higher capacity to generate firm adhesions and stable conjugates with antigen-presenting DCs via LFA-1, compared to naïve T cells. This ability may likely reside in the higher affinity states of LFA-1 maintained by activated and differentiated T cells [32]. In addition, the local secretion of DC-produced inflammatory chemokines might provide these activated T cells with co-

stimulatory integrin activating signals [33, 34]. Notably, PI3K $\delta$  signaling has been implicated in the acquisition of high affinity LFA-1 states by TCR activated T cells inside murine lymph nodes [32]. Whether **PI3K $\delta$**  is directly involved in TCR mediated inside-out signaling to LFA-1 alone or in conjunction with **GPCRs** such as CCR5, CXCR3 or XCR1 remains an interesting possibility that merits further attention.

Of note, in the absence of DC-expressed ICAM-1, murine CD8<sup>+</sup> T cells can still proliferate normally and differentiate into short lived cytotoxic T lymphocytes (CTLs) [35]. Thus, a link between the ability of T cells to firmly arrest on DCs in an ICAM-1-dependent manner and the generation of long lasting memory was suggested, but could not be established due to the use of total *Icam1*<sup>-/-</sup> mice rather than of conditional DC-specific ICAM-deficient animals in this milestone study [35]. Thus, the precise contribution of DC-expressed ICAM-1 and of T cell-expressed LFA-1 to T cell differentiation and acquisition of long-lasting memory remains questionable. Another open question is if, and under what priming conditions (e.g., the strength of the TCR signal) does an initial T cell division actually become asymmetric [36] and whether individual daughter T cells with different cellular compartmentalization and affinity states of LFA-1 can differentially engage ICAM-1 on antigen-presenting DCs after such divisions [37]. It is also unclear if the differentiation of effector T cells into effector memory subsets via CD40-CD40L signaling [38] does utilize a unique subset of highly adhesive LFA-1 that can bind DC ICAM-1, independently of TCR-dependent inside-out signaling.

**LFA-1/ICAM-1 dependent T cell communication**

Following activation, antigen-specific T cells surrounding APCs also interact with themselves [15, 39-41] (Figure 2). These T cell:T cell (T-T) interactions are organized into multifocal synapses in CD4<sup>+</sup> T cells [39] mediated by LFA-1 and ICAM-1 interactions, with dwell times between 5 to 10 minutes in mouse CD8<sup>+</sup> T cells [42]. T-T cell synapses promote the targeted delivery of cytokines such as **IL-2** and **IFN- $\gamma$**  to other T cells [39, 42, 43] and provide a platform for co-incidental signaling. Notably, LFA-1 and IFN- $\gamma$  signaling at the T-T interface results in the integration of both downstream signaling pathways, where IFN- $\gamma$ -induced STAT1 phosphorylation is enhanced by integrin-mediated

37 activation of Src kinases [43]. Moreover, the crosstalk between LFA-1 and IFN- $\gamma$  is complex and not  
38 limited to T-T interactions. Constitutive ICAM-1 expression on naïve T cells is enhanced upon IFN-  
39  $\gamma$  treatment [44]; also, stimulation of mouse splenocytes by secreted interferon stimulated gene 15  
40 (**ISG15**) *in vitro* induces IFN- $\gamma$  secretion upon binding to LFA-1 [45]. This suggests that ICAM-1 and  
41 LFA-1 have co-stimulatory properties in addition to adhesive functions when engaged at the T-T  
42 interface, as hypothesized in earlier studies [46]. It is however unclear whether LFA-1/ICAM-1  
43 signalling is bi-directional at T-T synapses, and whether it could also act in cis.

44 T cells use LFA-1/ICAM-1 dependent interactions to regulate their expansion. ICAM-1  
45 deficient T cells, which can interact with DCs but not with other T cells, display decreased cell  
46 number compared to wild-type T cells following TCR activation in vitro or in mice infected by  
47 Lymphocytic choriomeningitis virus (LCMV), *Listeria monocytogenes* (LM) or immunized with DC-  
48 bearing antigen [42, 47, 48]. Mechanistically, ICAM-1-mediated T cell clustering can regulate T cell  
49 survival following TCR triggering in vitro or in vivo in mice following LCMV infection [31], in part by  
50 increasing Bcl-2 expression [49]. The function of homotypic contacts in T cell expansion and survival  
51 is most likely linked to mechanisms similar to **quorum sensing** – a regulatory mechanism based  
52 on the ability of cells to detect specific extracellular factors they produced in order to sense their  
53 population size [50]. Mathematical modelling shows that CD8<sup>+</sup> T cells use nested antagonistic IL-2  
54 and CD80/86-CTLA-4-mediated feedback mechanisms dependent on contacts to regulate their  
55 expansion based on cellular density. Modelling has been confirmed in a DC-free system, where  
56 CD8<sup>+</sup> T cells primed with cognate peptide still clustered and proliferated in an ICAM-1 dependent  
57 manner. Similarly, expansion of adoptively transferred ICAM-1 deficient T cells following LCMV  
58 infection in mice was reduced compared to control cells, suggesting that T-T interactions improve  
59 survival [47]. T cell interactions can also result in the intercellular transfer of membrane components,  
60 including TCRs, through **trogocytosis** in in vitro co-culture system of activated CD8<sup>+</sup> T cells [51],  
61 potentially amplifying T cell expansion.

62 T cells can also directly interact with each other to co-regulate their differentiation. For  
63 instance, LFA-1 stimulation of human CD4<sup>+</sup> T cells by recombinant ICAM-1 during TCR priming in

vitro promotes **Th1 polarization**, detected by IFN $\gamma$  production of CD4 $^{+}$  T cells [52]. Furthermore, in mice, LFA-1 dependent-CD4 $^{+}$  T cell interactions likely contribute to antigen presentation between T cells, resulting in their activation, proliferation, and differentiation. CD4 $^{+}$  T cells can take up peptide/MHC complexes from APCs and present them to cognate T cells following priming in vitro and in vivo, demonstrated by an elegant approach using a TCR transgenic T cells and APCs of different MHC haplotypes [53, 54]. A similar T-T activation model has been proposed for humans T cells, which can express molecules required for antigen presentation (increased ICAM-1, CD80, CD86, MHC II) following in vitro priming [53, 54]. As T cells require LFA-1/ICAM-1 to form T-T synapses [55-57], it is likely that LFA-1 and ICAM-1 interactions participate to T-T antigen presentation. *In vitro*, T-T interactions induced by TCR priming induces the generation of CD4 $^{+}$  T cells with a **regulatory phenotype**, which is inhibited by LFA-1 blocking antibodies [58]. Blocking overall T-T contacts *in vitro* using a semipermeable membrane and CTLA-4 deficient T cells provided evidence that mouse CD8 $^{+}$  T cell expansion and differentiation also relies on contacts and CTLA-4. CTLA-4 deficient T cells fail to activate the Hippo signaling pathway and to express Blimp-1, leading to the suppression of T cell expansion and differentiation [59]. As such, T cell interactions control and link the magnitude of clonal expansion to terminal differentiation. However, the direct function of ICAM-1 on CD8 $^{+}$  T cell differentiation and function is unclear. ICAM-1 KO CD8 $^{+}$  T cells display increased IFN- $\gamma$  production compared to their WT counterparts following TCR triggering *in vitro*; and increased effector differentiation following Incomplete Freund Adjuvant (IFA)-OVA immunization in mice, as evidenced by expression of the effector marker **KLRG1** [48]. In contrast, in acute LCMV and LM infection models and in a vaccination model relying on anti-Dec205 delivery of antigen, ICAM-1 deficient CD8 $^{+}$  T cells display decreased effector differentiation based on the phenotypic markers CD44 and CD62L [42, 47] and decreased IFN- $\gamma$  production *in vivo* relative to control CD8 $^{+}$  T cells [42]. While the discrepancy between models is not totally understood, it likely reflects the ability of LFA-1 and ICAM-1 dependent interactions to support multiple/distinct signals, leading to potentially contrasting outcome.

Finally, T cell contacts have been described between different T cell subsets. Memory and naïve CD8<sup>+</sup> T cells can interact during priming, leading to faster differentiation of naïve cells into effector memory T cells. This requires Fas, as recombinant FasL treatment during in vitro TCR priming increases the differentiation of naïve T cells in effector memory T cell subset and enhances IFN- $\gamma$  production [60]. It has been hypothesized that this process synchronizes the functional and transcriptional state of naïve T cell progeny with that of memory cells [60]. CD4<sup>+</sup> and CD8<sup>+</sup> T cells have also been shown to directly interact using 2-photon microscopy in mice immunized with APCs [61, 62]. CD8<sup>+</sup> T cells directly activate CD4<sup>+</sup> T cells with peptide/MHC-II acquired from their activating APC via trogocytosis. Activated CD4<sup>+</sup> T cells in turn skew CD8<sup>+</sup> T cells towards long term memory. CD8<sup>+</sup> T cells that were “helped” in vitro display enhanced recall expansion upon subsequent challenge in vivo with LM compared to “helpless” CD8<sup>+</sup> T cells [61, 62], indicating that direct interaction between CD8<sup>+</sup> and CD4<sup>+</sup> T cells may contribute to **CD4<sup>+</sup> T cell help**.

Overall, LFA-1/ICAM-1 interactions between T cells create a platform allowing for signal integration, where the functional outcome of T cell clustering might depend on the other signals shared within clusters. This is important to tightly control T cell differentiation and tailor it to the injury. In addition, T-T interactions enable the regulation of T cell response at the population level, which we speculate is crucial for the maintenance of tolerance.

### **The LFA-1–C3–CD46 axis: a driver of human Th1 responses**

In CD4<sup>+</sup> T cells, LFA-1 is mostly associated with the induction of Th1 responses [63]. TCR stimulation triggers the active form of the LFA-1 integrin complex [8] promoting LFA-1 outside-in signaling (Figure 1) and ultimately the activation of key enzymes, the spatial control of transcription factor (TF) movement, cytoskeletal remodeling [64, 65], and changes in gene expression – all events underlying successful Th1 induction both in mice and in human CD4<sup>+</sup> T cells [66, 67] (Figure 3). LFA-1 further enforces the Th1 program by concurrently inhibiting Th2 differentiation via GATA3 suppression in mouse CD4<sup>+</sup> T cells [68, 69].



LFA-1-induced signals also integrate with other effector systems to fine tune T cell activity. For example, LFA-1 activates GSK-3 $\beta$  and the  $\gamma$ -secretase complex, which subsequently cleaves Notch-1 from the human CD4<sup>+</sup> T cell surface [70]. The released intracellular signaling domain of Notch-1 (NICD) translocates to the nucleus and mediates the expression of the Notch-1 target genes, *HEY1* and *HES1*. These in turn reinforce Th1 induction in human CD4<sup>+</sup> T cells by supporting *TBET*, *IFNG* and *IL2* expression [71, 72] (Figure 3).

LFA-1 also engages another evolutionary old effector system for Th1 responses, namely the intracellular complement system (the complosome). LFA-1 activation by ICAM-1 on *in vitro* cultured human CD4<sup>+</sup> T cells triggers high gene expression of the central complement component C3 in an AP-1-dependent fashion [73]. Concurrent stimulation of the TCR in such *in vitro* LFA-1 stimulated human CD4<sup>+</sup> T cells then leads to a rapid increase in C3 protein expression, its proteolytic activation into bioactive C3a and C3b, and the translocation of C3b to the T cell surface [73]. Here, intrinsic C3b engages the human-specific complement receptor CD46 and the intracellular signaling domain(s) of CD46 (termed CYT-1 and CYT-2 [74]) – similar to Notch-1 – are processed by  $\gamma$ -secretase. They then undergo nuclear translocation, as shown in freshly isolated human CD4<sup>+</sup> T cells [75-77]. The CD46 tails induce the expression of genes encoding glucose and amino acid transporters and ultimately mediate mammalian target of rapamycin complex 1 (mTORC1) activation, as well as high glycolysis and oxidative phosphorylation (OXPHOS). High thresholds of glycolysis are specifically needed for the translation of *IFN* $\gamma$  mRNA in primary human CD4<sup>+</sup> T cells as reduction in glycolysis (for example, via usage of glycolysis inhibitors or due to abrogation of CD46 expression) in these cells translates into reduced IFN- $\gamma$  production and Th1 induction [73, 75, 78-80] (Figure 3).

LFA-1-induced 'C3 licensing' is also a feature of human CD8<sup>+</sup> T cells and monocytes, supporting their normal effector activity, as CTLs or monocytes lacking normal LFA-1 expression levels have a defect in IFN- $\gamma$  secretion and cytotoxic activity or IL-1 $\beta$  production, respectively [73]. As a consequence, **CD46-deficient patients**, or patients with reduced C3 secretion capacity, present with ablated Th1/CTL responses and can suffer from recurrent opportunistic infections of

the upper respiratory tract [81]. Similarly, patients with mutations in the *ITGB2* gene who suffer from **leukocyte adhesion deficiency 1** (LAD-1) cannot initiate increases in intracellular C3 beyond basal concentrations, and specifically exhibit reduced Th1 and CTL responses *in vitro*, as well as harbor monocytes that produce reduced IL-1 $\beta$  relative to monocytes from healthy donors with normal LFA-1-mediated C3 licensing [73]. Thus, although still hypothetical, the inability of LAD-1 T cells to upregulate C3 and engage CD46 in an autocrine fashion might be a common link with CD46-deficient T cells: Th1 immunity is defective although these T cells proliferate normally and are able to produce ample Th2 and Th17 cytokines [71]. Furthermore, *in vitro* CD46 engagement on human CD4<sup>+</sup> T cells with antibodies to CD46 can mimic the key activities of LFA-1 during CD4<sup>+</sup> T cell stimulation (Figure 3), including activation of cellular kinases underlying human CD4<sup>+</sup> T cell activation, such as AKT [82], contribution to immunological synapse formation [83], control of Notch 1 signalling in human CD4<sup>+</sup> T cells [84], and Th1 induction, by temporally controlling expression of lineage-skewing cytokines such as IL-10 and/or TGF- $\beta$  in human CD4<sup>+</sup> T cells [85-87]. Moreover, LFA-1 engagement appears to parallel the central metabolic reprogramming profile triggered by direct CD46 co-stimulation, namely, inducing increase in glycolysis as lack of LFA-1 engagement on human CD4<sup>+</sup> T cells reduces glycolysis and T cell activation and migration [88, 89]. It should be noted however, that it is not clear if and how exactly the defective Th1 response from LAD-1 patients observed *in vitro* contributes to disease pathology *in vivo*. LAD-1-associated oral lesions are now considered induced by uncontrolled Th17 and IL-17-producing innate lymphoid cell activities [90]. Th1 cells negatively control Th17 cells in mice during mucosal inflammation [91, 92] and we noted that *in vitro* normalization of intracellular C3 and IFN- $\gamma$  in CD4<sup>+</sup> T cells from LAD-1 patients simultaneously reduced their hyperactive Th17 responses. We, thus, favor the hypothesis that reduced local Th1 immunity may contribute to detrimental Th17 tissue responses in LAD-1.

Overall, current data indicate that the integrin-complement connection seems to jointly control central cell metabolic events in human CD4<sup>+</sup> T cells – which aligns with the growing understanding that LFA-1 might be an important upstream regulator of leukocyte metabolism, although this warrants further and robust investigation.

The LFA-1–C3–CD46 axis is a recent discovery and there are many questions surrounding this new functional connection between an integrin and the complement system. For example, it is unclear when LFA-1 engagement on T cells is most important for productive C3 generation and Th1 induction. We speculate that this might occur during extravasation of T cells into lymph nodes, or later on, where emigrating T cells can receive additional TCR triggering signals from APCs because we noted that C3 gene expression triggered by LFA-1 alone returns to base-levels by about 12 hours post stimulation without an incoming TCR signal [73]. Thus, the cognate T cell-APC interaction likely sustains C3 amounts and endows cells with the ability to acquire a Th1 phenotype, although this remains to be tested. However, because CD46 activation can increase ICAM-1 expression on human CD4<sup>+</sup> T cells [73] and can redistribute key LFA-1 ligands into the T cell **uropod** [93], facilitating T cell:T cell interactions [94], it is feasible that LFA-1–ICAM-1 interactions underlying homotypic interactions of expanding effector cells in the lymph node might also be functionally important for sustained C3 licensing (Figure 2). Dissecting these possibilities will not be straightforward: CD46 is a human-specific protein as rodents do not express CD46 in somatic tissues and the murine ortholog has not been identified [95]. This suggests that integrin and complement crosstalk modulating T cell biology may have significant species-specific aspects – and this should be taken into consideration when studying Th1 differentiation and function in mouse models.

Of note, T cells can be long-living and spend a considerable proportion of their life span in peripheral tissues such as lung, skin, intestinal tract, etc. in both mice and humans [96]. For instance, high C3 expression has been recently reported as a cardinal feature of human CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the lung [73]. Furthermore, CD8<sup>+</sup> T cell-expressed LFA-1 is required for liver-resident CTLs to remain in hepatic sinusoids in mice as inhibition of LFA-1 prevented retention of CTLs in the liver [97]. Also, depletion of CD44<sup>+</sup> LFA-1<sup>+</sup> liver-resident CD8<sup>+</sup> T cells strongly reduced immune clearance of hepatitis B virus in a mouse model of infection [96]; however, the authors did not evaluate the effects of just blocking LFA-1 on hepatitis B virus infection. Collectively, these findings suggest that LFA-1 outside-in signaling to the complosome might contribute to regulating immune cell memory

in an organ-specific manner, although these mechanistic insights will require further rigorous investigation (Figure 4).

### **LFA-1 on CTLs reaching effector sites**

LFA-1 plays also important roles in the killing of virally-infected cells or tumor cells by CTLs (Figure 4). CTL – target cell interactions in mice are often short lived, lasting only minutes before the CTL induces apoptosis in the target and moves on to the next target, and so are more akin to kinapses [98]. In the cytolytic synapse of CTLs, LFA-1 surrounds the TCR-rich center and the secretory domain, forming an enclosed space, also described as a gasket that prevents secreted granules from leaking out and killing non-infected neighboring cells [99]. In addition, injection of ICAM1-blocking antibodies into B16-VEGFC melanoma bearing mice showed that ICAM-1-mediated homotypic interactions between tumor infiltrating lymphocytes can serve as an immune retention mechanism keeping activated CD8<sup>+</sup> T cells within the tumor [100]. The importance of LFA-1 is also highlighted by a series of microscopy experiments that showed that Galectin 3 – a lectin secreted by many tumor cells and macrophages – can interfere with both LFA-1 recruitment to the IS and affinity regulation [101] and consequently CTL effector functions. Treatment of human CD8<sup>+</sup> tumor infiltrating lymphocytes (TILs) obtained from tumors of different histological origin with an anti-galectin-3 antibody or a galectin competitive binder, such as LacNAc, resulted in the detachment of surface galectin-3 from the T cells and led to improved TIL spreading over a superantigen-pulsed target cell line, as well as actin clearance and ultimately increased degranulation, secretion of IFN- $\gamma$  and target cell killing when compared to untreated TILs [101]. The underlying mechanism is only poorly understood but might be due to LFA-1 being trapped in glycoprotein-galectin lattices that cause a physical barrier and thereby lead to reduced recruitment of LFA-1 to the IS [101]. The above described defects in IS formation were recapitulated following partial blocking of LFA-1 with function blocking antibodies, suggesting that LFA-1 outside-in signaling might synergize with TCR induced inside-out signaling and participate in actin remodeling in CTLs [101]. Notably, the C-type lectin domain containing glycoprotein Layilin is highly expressed in CD8<sup>+</sup> TILs isolated from human melanoma biopsies [102]. Deleting Layilin through CRISPR-Cas9 electroporation in human CD8<sup>+</sup> T

cells led to strongly reduced target cell killing compared to wildtype CD8<sup>+</sup> TILs in an *in vitro* killing assay [102]. The study showed that Layilin interacts with LFA-1 through talin and promotes the high affinity open confirmation of LFA-1 as measured by the binding of an anti-LFA-1 antibody that specifically recognizes the activated conformation of LFA. Indeed, a point mutation in Layilin's talin binding domain leads to reduced LFA-1 mediated adhesion relative to control T cells, even in the presence of Mn<sup>2+</sup>, which directly activates LFA-1; this suggests that the layilin adaptor can modulate post-ICAM-1 binding clustering and outside in signaling of LFA-1 [102], which may make it a target for overexpression in adoptive T cell transfer therapies as it may augment the T cell response.

From another angle, CTLs exert considerable mechanical forces on their target cells in an actin cytoskeleton dependent manner which improves the efficiency of target cell membrane perforation and hence killing [103, 104]. During spreading, CTLs exert pushing forces in an outward direction on their targets, but this quickly reverses, and the cells begin to squeeze inwards [103]. Experiments with PDMS micropillars that deflect under the force of CTLs seeded on top of them showed that pillar deflections, which are a readout for forces generated by the CTL, are enriched in the regions of the **pSMAC** and **dSMAC**, where LFA-1/ICAM-1 interactions occur within the IS [103]. Similar enrichment results were obtained by **traction force microscopy**, suggesting that actin dynamics and LFA-1/ICAM-1 interactions might contribute to driving synaptic force generation [105]. Moreover, experiments with the pH-sensitive fluorescent reporter pHluorin-LAMP1 that localizes to cytotoxic granules and becomes visible only after fusion with the plasma membrane [106], showed that degranulation occurs preferentially in close proximity to LFA-1 near the base of actin rich force generating cell membrane protrusions that were dependent on cytoskeletal regulators **WASP** and **Arp2/3** [107]. In summary, LFA-1 clearly contributes and is indeed necessary for the formation of a functional cytotoxic synapse and consequently CTL effector response.

**Regulation of LFA-1 signaling at peripheral effector sites; lessons from PTPN22 mutant lymphocytes**

Emerging concepts have begun to shed light on how altering thresholds of LFA-1 signaling can profoundly dysregulate T cell function [108]. Knowledge of proximal signaling intermediates downstream of LFA-1, notably src and syk kinases [2], raised the possibility that the protein tyrosine phosphatase PTPN22, known to regulate membrane proximal TCR signaling by targeting these substrates [109], might also regulate LFA-1 signaling. To study the impact of PTPN22 on LFA-1 signals independently of its effects on TCR dependent inside out signaling, engagement of LFA-1 expressed by T cell blasts by immobilized Fc:ICAM-1 constructs was found to be necessary and sufficient to phosphorylate Lck, ZAP-70 and Vav, as demonstrated by immunoblotting experiments [108]. In T cells deficient in PTPN22, or expressing the loss-of-function PTPN22-R620W mutant, these membrane proximal phosphorylation events were increased when compared to wild type T cells [78]. Co-immunoprecipitation experiments in Lck deficient Jurkat T cells indicated that the phosphatase likely interacts with the LFA-1 tail in an Lck-dependent manner [78]; these findings aligned with the colocalization of PTPN22 and its substrates demonstrated by both confocal and **TIRF microscopy** at the **leading edge** of polarized T cells migrating on immobilized ICAM-1 *in vitro*.

If PTPN22 is a negative regulator of LFA-1 signaling, then it follows that the conformation and distribution of LFA-1 molecules on the surface of phosphatase mutant T cells might be distinct from those of wild type T cells. As a direct consequence of increased LFA-1 signals, LFA-1 was found to be more highly clustered on the membrane of human T cells expressing loss of expression/function PTPN22 mutants [108]. Functionally, and relative to PTPN22 sufficient T cells, human T cells expressing loss of function PTPN22 mutants exhibit increased adhesiveness under **shear flow**, and faster migration [108]. Likewise murine CD4<sup>+</sup> T cells from *Ptpn22* deficient mice, exhibit increased ICAM-1/LFA-1 dependent synapse formation [108]. This was manifest by increased density of its counter-ligand ICAM-1 in supported lipid bilayers at the IS, when compared to wild type CD4<sup>+</sup> T cells [110]. It should be noted that increased adhesion has been observed for both effector and regulatory T cell subsets, with functional consequences [110, 111]. For example, increased conjugates were observed between *Ptpn22*<sup>-/-</sup> **OTII T cells** and OVA peptide-pulsed DC, when compared to wild type OTII T cells, and substantially increased Th1 responses, as evidenced

from peptide-specific IFN $\gamma$  production [110]. On the other hand, increased adhesive properties of *Ptpn22*<sup>-/-</sup> regulatory T cells were reflected in more potent regulatory function *in vivo*, in a colitis adoptive transfer model [111]. This enhanced regulatory function may explain the lack of spontaneous disease in *Ptpn22* deficient mice [81].

Of clinical relevance, a spontaneously occurring mutation of PTPN22, PTPN22-R620W is associated with a wide range of autoimmune diseases in humans, such as type 1 diabetes, rheumatoid arthritis and lupus [112, 113]. Based on the effects of the PTPN22 phosphatase on TCR and LFA-1 signaling, the impact of PTPN22 mutations in functional terms, provides a useful framework for understanding why genetic variation of PTPN22 is associated with as many as sixteen distinct autoimmune syndromes [83]. Exploiting the OT-I response to OVA peptides, and the availability of peptide variants eliciting weaker TCR signals, increased LFA-1 dependent responses were observed in *Ptpn22*<sup>-/-</sup> CD8<sup>+</sup> T cells stimulated by weak agonists, when compared to wild type T cells. This was related to early events, since co-culture of T cells with peptide loaded APCs led to increased frequencies of T cell:APC conjugates, as well as activation of **Rap1**, increased T cell adhesion to ICAM-1 expressing monolayers under shear flow, and increased **homeostatic T cell proliferation** and IFN $\gamma$  production compared with wild type CD8<sup>+</sup> T cells [114]. Collectively, these murine and human data suggest that PTPN22 can fine-tune the TCR-mediated discrimination of low avidity self-antigens, such as those that are relevant for certain autoimmune diseases. We propose that the disease associated PTPN22 variant may achieve this by reducing thresholds of LFA-1 dependent T cell activation at the T cell:APC interface, but also by augmenting costimulatory and migratory functions following outside-in integrin signals.

## **Novel regulators of LFA-1**

Chemokine and TCR-triggered inside-out activation of LFA-1 has been extensively reviewed elsewhere [2]. However, several new key regulators have recently been discovered. One of them is the serine/threonine kinase **WNK1** – identified in an RNAi screen as a key negative regulator of LFA-1-dependent adhesion of Jurkat T cells to B cells *in vitro* [115]. WNK1 is the only Wnk1-family

member expressed in both primary mouse CD4<sup>+</sup> and CD8<sup>+</sup> T cells ,and has been mostly studied for its role in controlling ion homeostasis [116]. *Wnk1*<sup>-/-</sup> naïve mouse CD4<sup>+</sup> T cells bind more strongly to endothelium and soluble ICAM-1 complexes and transmigrate slower into lymph nodes than heterozygous *Wnk1*<sup>+/-</sup> CD4<sup>+</sup> T cells. Once inside of lymph nodes, these T cells continue to migrate more slowly than *Wnk1*<sup>+/-</sup> T cells [115]. WNK1 is activated by TCR, CCR7 [115] and CXCR4 [117] signaling through AKT and PI3K and negatively controls Rap1 GTP-loading, a crucial step in activating LFA-1 [115]. Of note, the chemokine-induced switch to high LFA-1 affinity in Jurkat cells as measured with a reporter mAb is not affected by loss of WNK1, suggesting that WNK1 may control adhesion strengthening events such as LFA-1 micro and macroclustering [115]. Another recently discovered negative regulator of LFA-1 is **MAP4K4**: Genetic deletion of MAP4K4 in an inducible mouse knockout model led to decreased phosphorylation of **ERM proteins** in primary mouse CD8<sup>+</sup> T cells as measured by flow cytometry as well as increased conjugation to APCs and adhesion to ICAM-1 [118]. As a consequence, MAP4K4 deficiency resulted in increased CD8<sup>+</sup> T cell priming, activation, proliferation, cytokine production and cytotoxicity. Furthermore, several novel positive regulators of high affinity LFA-1 have also been discovered, such as the serine/threonine kinases **NDR1** and **NDR2** [119, 120]. Immunoblotting studies showed that NDR1 becomes phosphorylated and activated through a Rap1 and MST1/MST2-dependent pathway in primary mouse CD4<sup>+</sup> T cells and plays part in recruiting kindlin3 to the IS in naïve CD4<sup>+</sup> T cells and in **cSMAC** formation and high affinity binding of LFA-1 to ICAM-1 on **supported lipid bilayers** [119]. Additional details were then reported for NDR2 in a further immunoblotting study using both Jurkat cells in which NDR2 expression was silenced and primary mouse *Ndr2*<sup>-/-</sup> CD4<sup>+</sup> T cells. This study showed that NDR2 phosphorylates Filamin-A in response to TCR signaling, facilitates dissociation of Filamin A from LFA-1 and allows the binding of talin and Kindlin3, which stabilize the open high affinity conformation of LFA-1 [120]. It is likely that both NDR1 and NDR2 might act redundantly, although this has not been tested. The complexity of LFA-1 signaling was further highlighted in a recent study that used single molecule imaging to show the existence of a positive feedback mechanism in a mouse pro B cell line overexpressing human LFA-1 in which ligand binding of LFA-



1 induced transient interactions with talin-1 and the recruitment of Kindlin-3, which in turn led to a conformation change in LFA-1 and stable talin-1 binding {Kondo, 2021 #6525}. A direct consequence of this stabilization - although the mechanism isn't clear - was the further activation of the inside-out regulator Rap1, causing additional LFA-1 molecules to adopt the open high affinity conformation {Kondo, 2021 #6525}. While this was shown with a pro B cell line, it is likely that a similar mechanism will operate in T cell. Another example of a positive regulator is the actin binding protein **TAGLN2**, which physically associates with LFA-1 as shown through immunoprecipitation experiments and is required for the killing of ICAM-1-expressing target cells by primary mouse cytotoxic T cells (Figure 4), but not ICAM-1-deficient cells, suggesting a role in the LFA-1-dependent formation of cytolytic synapses [121, 122]. Lastly, a novel secreted ligand, ISG15, was recently found to bind to the extracellular domain of LFA-1 and induce outside-in signaling and the induction of IFN- $\gamma$  secretion in mouse splenocytes [45].

## Concluding remarks

LFA-1-ICAM interactions within extravascular compartments do not merely serve as an adhesive glue but provide co-stimulatory cues via LFA-1 outside-in signaling, fine tuning a wide range of immune effector phenotypes, including polarization into T cell effector subtypes and the formation of long-lasting memory. Furthermore, mechanical forces that are transduced from T cells and onto T cells through LFA-1-ICAM-1 interactions are likely to impact T cell functional outcomes, which is an area of intense investigation. Indeed, dysregulated LFA-1 signaling is relevant to disease pathology [108], where it can perturb the sampling of MHC-peptide complexes during early antigenic peptide recognition, affect homotypic cell to cell interactions [43], cell adhesion, migration speeds and transmigration [115], or modulate costimulatory functions [73] (see also Outstanding Questions).

There has been a long-standing interest in LFA-1 as a therapeutic target in the pharmaceutical industry, particularly during the early 2000s. Targeting LFA-1 directly – mostly with function blocking antibodies or small antagonist, however, turned out to be a blunt approach and

many of the clinical trials failed early on, due to severe side effects, that in parts may have been due to the very broad expression of LFA-1 on many immune cells. **A better** understanding of the signaling pathways downstream of LFA-1, however, may create opportunities to target only specific cell types to either suppress them, for instance in the treatment of autoimmune diseases or after transplantations, or to enhance their function to improve for instance T cell killing of virally infected or transformed cells.

### **Box 1: LFA-1, TCR, and the actin cytoskeleton**

The present consensus is that LFA-1 activation/signaling is not only mechanosensitive, but can also transduce forces and thereby activate mechanosensitive cytoskeletal adaptors. Underlying both of those is the direct connection and anchoring of activated LFA-1 to the actin cytoskeleton as well as LFA-1-induced actin polymerization and actomyosin contractility [123].

The IS was first described as a monofocal radial structure, but synapses between DCs and T cells are multifocal [124, 125] and specialized at priming and activating naïve and central memory T cells [126]. Of note, ICAM-1 on activated mouse DCs displays much less lateral mobility than on naïve DCs [124, 127], which allows activated LFA-1 to generate **catch bonds** with ICAM-1 [128]. As TCR signaling alone is insufficient to induce a switch in LFA-1 affinity, binding to ICAM-1 is necessary to stabilize high affinity LFA-1 states [129]. The **centripetal flow** of actin in T cells spreading over APCs moves LFA-1 inwards towards the center of the IS, which is opposed by ICAM-1 anchored to the cytoskeleton of the APC [130, 131]. This creates tension on LFA-1, thus inducing the high affinity open conformation [130, 131]. Hence, only when properly anchored to the DC cytoskeleton, can high affinity LFA-1-ICAM-1 bonds undergo stabilization by centripetal actomyosin driven forces generated by TCR signaling [130, 131].

This high affinity LFA-1 can then drive outside-in signaling [132] by recruiting Tiam [133], Kindlin-3 [134, 135], FAK [136, 137], Pyk [136] and other cytoskeletal regulators, eventually leading to RhoA and Rac1 activation [133, 138, 139] and further actin remodeling. Recently, one study [140] reported that CD4<sup>+</sup> T cells from **Crk-L** and **Crk** double knockout mice were spreading less efficiently

on surfaces coated with the LFA-1 substrate ICAM-1 and displayed less actin staining at the cell surface than wildtype CD4<sup>+</sup> T cells, indicative of impaired actin polymerization. Additional immunoblotting experiments showed that Crk proteins are required for the phosphorylation of **cCbl**, which likely has a role in the induction of actin polymerization upon LFA-1 signalling, and the force sensing adaptor protein **Cas-L**, which can act as a scaffold for additional signaling molecules in response to mechanical cues originating from the binding of T cells to other cell types [140]. To further delineate the contributions of TCR and LFA-1 signaling to actin dynamics [139] one group imaged human CD4<sup>+</sup> T cells on **micropatterned surfaces** on which immobile ICAM-1 and TCR stimulating anti-CD3 antibodies were physically separated. A microscopic analysis of the spreading of those T cells in conjunction with the use of the Arp2/3 inhibitor CK666 and the formin inhibitor SMIFH2 showed that actin polymerization downstream of the TCR was driven by the Arp2/3 complex and was extended by LFA-1-ICAM-1 engagement through the **formin FHOD1**, which led to enhanced myosin-dependent cytoskeletal tension resulting in increased T cell spreading and further TCR activation, suggestive of a positive feedback mechanism. Precisely how these biochemical circuits of TCR signaling to the cytoskeleton converge with LFA-1 outside-in signaling to the cytoskeleton and how mechanical forces regulate these processes is still not fully understood. New super-resolution microscopy tools might assist in dissecting the possible crosstalk between LFA-1 and these distinct TCR regulated effector molecules during distinct stages of IS formation.

#### **Figure 1: Multidimensional regulation of LFA-1 affinity in mice and humans**

LFA-1 is regulated by multiple extracellular and intracellular cues. In the low affinity state, the ligand binding domain is inaccessible to ICAM-1 [141]. Upon initiation of signals derived from G-protein coupled receptors and/or antigen T cell receptor (inside-out signaling), Rap1-GTP activation recruits signaling intermediates such as RAPL to the  $\alpha$ L subunit [142]. The resulting conformational change requires LFA-1 tail unclasping, facilitated by binding of two actin cytoskeletal adaptors, talin1 and Kindlin-3 to the  $\beta$ 2 subunit tail, stabilizing the high affinity state [141]. Binding of RIAM, talin, paxillin and vinculin to the cytoplasmic tails recruit additional intermediates forming a scaffold for interaction with cytoskeletal elements (outside-in

signaling). These cytoplasmic changes are transmitted to the head piece domain(s) and couple to additional LFA-1 rearrangements induced by ligand binding to the headpiece of LFA-1. They underpin a dynamic process of mechano-sensing based on ICAM and actin derived adhesive forces, propagated along the  $\beta 2$  subunit [8]. Extracellular ISG15, a ubiquitin-like secreted protein, can induce IFN- $\gamma$  expression by binding the  $\alpha L$  domain, thereby promoting outside-in signaling [45]. Negative regulators of outside-in signaling include the kinase WNK1 and phosphatase PTPN22 [108, 115]. For simplicity, micro- and macro-clustering of ICAM-occupied LFA-1 (which further facilitate adhesion and outside-in signaling) are not included in this scheme.

## **Figure 2: The LFA-1-ICAM axis in distinct phases of naïve murine T cell priming and differentiation in reactive lymph nodes.**

T cells use their LFA-1 to enter lymph nodes by arresting on ICAM-1 and ICAM-2 expressed by HEVs in response to chemokine signals (not shown). Initial naïve T cell activation by antigenic signals takes place by ICAM-1-independent serial encounters with LN and migratory DCs that present cognate antigenic peptide/MHC complexes following immunization or infection[35, 143]. During this phase the antigen-stimulated T cells undergo sequential activation switches that render their LFA-1 sensitive to inside-out activation by additional TCR signals. In phase 2, stable T-DC conjugates lasting for several hours take place and are mediated by DC ICAM-1[35]. Later on the daughter T cells use their LFA-1 to interact with ICAMs clustered on other daughter T cells, while encountering additional antigenic and co-stimulatory signals from resident DCs, migratory DCs and plasmacytoid DCs [56]. These signals are likely needed for T cell differentiation into effector T cells and central memory T cells and are ideally transmitted within LFA-1-stabilized immune synapses. DCs engaged by cognate antigen-activated T cells can also serve as bridges between TCR activated CD4<sup>+</sup> and TCR activated CD8<sup>+</sup>T cells and these clusters may also recruit polyclonal Tregs with highly activated LFA-1 to their vicinity [144]. These Tregs may help attenuate excessive T cell proliferation and differentiation. The extent of T cell differentiation depends on the type of pathogen, the distribution of antigens on distinct subsets of DCs, and may vary with the type of draining lymph node.

## **Figure 3: Simplified model of the LFA-1–C3–CD46 axis driving Th1 induction in human CD4<sup>+</sup> T cells.**

T cell receptor (TCR) stimulation on human CD4<sup>+</sup> T cells induces the inside-out mediated activation of LFA-1 (see Figure 1). LFA-1 in turn induces increased intrinsic C3 gene expression and protein generation – which we termed ‘C3 licensing’ [73]. TCR-triggered activation of intracellular C3 into C3a and C3b and rapid translocation to the cell surface triggers autocrine engagement of CD46 [75]. Processing of the intracellular domain(s) of CD46 (IC) induces several CD46-mediated events, such transcription factor activation, metabolic reprogramming, etc. required for Th1 induction [78]. It remains to be explored if LFA-1 can contribute to these events independently of CD46 and at what point LFA-1-mediated C3 induction may be functionally (most) important for Th1 induction (see text for details).

#### **Figure 4: T cell interactions at effector sites in the periphery.**

Postulated functions of LFA-1 in distinct immune synapses. Effector T cells egressing specific lymph nodes that drain sites of infection or vaccination home back to these tissues via interactions with ICAMs and other CAMs expressed by inflamed blood vessels (top left panel). CTLs use their LFA-1 for direct killing of various virus infected cells as well as of tumor cells that express cognate neoantigens (middle upper panel). Th1 CD4<sup>+</sup> effectors use their LFA-1 to engage with and help infected macrophages (right upper panel). At various sites of infections Th1 effectors and CTL may use their LFA-1 to undergo reprogramming by monocyte derived DCs (monoDCs) and classical DCs (cDCs) that cross present antigenic moieties they collect from infected epithelial cells via multiple cargo transfer mechanisms not discussed in this review (left lower panel). The effector T cells (e.g., Th1 and Th17 CD4<sup>+</sup> lymphocytes) use their highly activated LFA-1 also to communicate with infected and inflamed epithelial cells and other mesenchymal cells that elevate the high affinity LFA-1 ligand ICAM-1 in response to cytokine signals along with elevation of MHC-I and II (lower right panel). Currently, none of these postulated functions of LFA-1 have been demonstrated *in vivo* due to lack of genetic models with cell type specific knockouts of either LFA-1 or ICAM-1 at effector sites.

#### **Glossary:**

**APC**, Antigen Presenting Cell, presents processed antigenic peptides on MHC class I or II to T cells.

**Arp2/3**, a protein complex that nucleates branched actin filaments.

70 **Avidity**, increased adhesion of LFA-1 due to receptor and ligand clustering

71 **Catch bond**, a noncovalent bond whose lifetime increases with applied tensile forces.

72 **Cas-L**, a CRK-associated substrate-related protein, also called Enhancer of filamentation 1, a

73 docking protein implicated in cell adhesion.

74 **cCbl**, E3 ubiquitin-protein ligase CBL, a negative regulator for tyrosine kinases.

75 **CD46-deficient patients**, individuals lacking cell surface expression of CD46. They develop

76 hemolytic uremic syndrome and mount reduced Th1 and CTL responses.

77 **Crk-L**, cytosolic adaptor protein which regulates cell adhesion, spreading, and migration.

78 **CD4<sup>+</sup> T cell help**, process by which CD4<sup>+</sup> T cells promote clonal expansion and effector and memory

79 differentiation of CD8<sup>+</sup> T cells

30 **cSMAC, central Supramolecular Activation Cluster**, central region of the immunological

31 synapse, contains the TCR- and associated signalling molecules

32 **DC, Dendritic Cell**, a major professional antigen presenting cell type, that processes proteins and

33 presents their peptides in cognate MHC complexes recognized by TCRs on naïve T cells.

34 **dSMAC, distal Supramolecular Activation Cluster**, outer region of the immunological synapse,

35 enriched in actin and negative regulators of TCR signalling such as CD45.

36 **FHOD1**, a formin family protein, highly expressed in T cells.

37 **Formins**, a family of actin nucleating proteins polymerizing linear actin filaments.

38 **FRC**, Fibroblastic Reticular Cell, the main stromal cell in lymph nodes and the white pulp of the

39 spleen.

30 **GPCR**, G-protein Coupled Receptor, a member of the largest group of membrane receptors, a

31 subfamily of which functions as chemokine and chemoattractant receptors.

32 **Homeostatic T cell proliferation**, T cell proliferation in the absence of antigen, particularly when T

33 cells are transferred into hosts lacking T cells.

04 **Kinapse**, a short-lived adhesive contact between a T cell and APC driven by a cognate antigen-  
05 TCR interaction

06 **KLRG1**, Killer cell lectin-like receptor subfamily G member 1

07 **LAD-1**, leukocyte adherence deficiency type 1, an immunodeficiency disease caused by loss or  
08 mutations in the CD18 integrin chain.

09 **LFA-1**, Leukocyte Function-Associated Antigen 1, also called CD11a/CD18 or  $\alpha L\beta 2$ , a major integrin  
10 adhesion receptor of all leukocytes.

11 **ICAM-1 and ICAM-2**, Intracellular Adhesion Molecule 1 and 2, high and low affinity ligands of LFA-  
12 1.

13 **IFN- $\gamma$** , Interferon  $\gamma$ , a cytokine that inhibits viral replication, activates numerous immune cells,  
14 induces MHC expression and enhances antigen presentation.

15 **ISG15**, Interferon Stimulated Gene 15, a small, secreted protein released in response to type 1  
16 interferons, recently shown to bind the extracellular domain of LFA-1 and induce outside-in  
17 signaling.

18 **IL-2**, Interleukin 2, a cytokine required for T cells proliferation, survival and acquisition of effector  
19 functions.

20 **IS, Immunological Synapse**, structured signaling platform between different immune cells as well  
21 as between cytotoxic cells and their targets.

22 **MAP4K4**, Mitogen-activated protein kinase kinase kinase kinase 4, required for adhesion, migration,  
23 proliferation.

24 **NDR1 and NDR2**, Nuclear Dbf2-Related 1 and 2, serine/threonine kinases, activated by the Rap1  
25 signaling cascade through RAPL and Mst1/Mst2.

26 **PI3K $\delta$** , Phosphatidylinositol 4,5-bisphosphate 3-kinase, activated in response to TCR signalling and  
27 involved in LFA-1 activation, T cell differentiation and proliferation

18 **pSMAC, peripheral Supramolecular Activation Cluster**, region of the immunological synapse,  
19 where LFA-1 and talin preferentially localize to.

20 **PTPN22**, Protein tyrosine phosphatase non-receptor type 22, negative regulator of multiple src and  
21 Syk family kinases.

22 **Quorum sensing**, a form of sensing mechanism whereby cells have the ability to detect and  
23 respond to cell population density by gene regulation.

24 **Rac1**, a small GTPase involved in controlling actin dynamics.

25 **Rap1**, a small GTPase, required for LFA-1 avidity regulation.

26 **Regulatory phenotype**, T cells (mainly CD4+) that express the transcription factor FoxP3 and  
27 regulate or suppress other cells in the immune system. They are important to prevent autoimmune  
28 diseases.

29 **RhoA**, a small GTPase involved in controlling actin dynamics.

30 **Shear flow**, force created by the blood flow, which T cell need to overcome to adhere to blood  
31 vessels and to migrate into tissues.

32 **TAGLN2**, Transgelin 2, an actin binding protein.

33 **Th1 polarization**, CD4<sup>+</sup> T cells that produce proinflammatory cytokines, the main being IFN- $\gamma$ .  
34 Signals leading to Th1 polarization include IL-12 at the time of priming.

35 **Traction force microscopy**, method to measure mechanical forces on the surface of cells.

36 **TIRF microscopy**, total internal reflection microscopy, particular useful to interrogate the contact  
37 site between a cell and a glass coverslip.

38 **Trogocytosis**, membrane transfer between live cells that occurs following conjugation

39 **Uropod**, the posterior protrusion of a polarized cell during cell migration.

40 **WNK1**, With No K (Lysine) 1, a serine/threonine kinase that regulates T cell adhesion and migration.

41 **WASP**, Wiskott Aldrich Syndrome Protein, actin nucleation promoting factor for Arp2/3 complex.

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## Declaration of Interests

The authors declare no competing interests.

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