T-cell integrins: more than just sticking points

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Summary

T cells use integrins in essentially all of their functions. They use integrins to migrate in and out of lymph nodes and, following infection, to migrate into other tissues. At the beginning of an immune response, integrins also participate in the immunological synapse formed between T cells and antigen-presenting cells. Because the ligands for integrins are widely expressed, integrin activity on T cells must be tightly controlled. Integrins become active following signalling through other membrane receptors, which cause both affinity alteration and an increase in integrin clustering. Lipid raft localization may increase integrin activity. Signalling pathways involving ADAP, Vav-1 and SKAP-55, as well as Rap1 and RAPL, cause clustering of leukocyte function-associated antigen-1 (LFA-1; integrin αLβ2). T-cell integrins can also signal, and the pathways dedicated to the migratory activity of T cells have been the most investigated so far. Active LFA-1 causes T-cell attachment and lamellipodial movement induced by myosin light chain kinase at the leading edge, whereas RhoA and ROCK cause T-cell detachment at the trailing edge. Another important signalling pathway acts through CasL/Crk, which might regulate the activity of the GTPases Rac and Rap1 that have important roles in T-cell migration.

Key words: Integrin, Leukocyte function-associated antigen-1, LFA-1, αLβ2, Adhesion, Migration

Introduction

Unchallenged T cells recirculate between the blood and the lymph nodes, awaiting encounters with antigen-presenting cells (APCs) (reviewed by Bradley, 2003). For T-cell stimulation to occur, the T cell must come into contact with an APC that has recently arrived from an infected peripheral tissue, such as skin or gut, where it has ingested viruses or bacteria, giving rise to the pathogen peptide recognized by the specific T-cell receptor (TCR). Following successful interaction between the T cell and APC, the T cell rapidly proliferates, becomes able to access nonlymphoid tissues by expressing a different set of 'homing' receptors and differentiates into an effector T cell. These short-lived T cells kill virus-infected cells, activate macrophages to kill ingested bacteria and activate B cells to produce antibodies. Some mature into memory T cells, which lodge for long periods in the spleen and at the site of the first infection, ready to respond rapidly if the infection re-occurs.

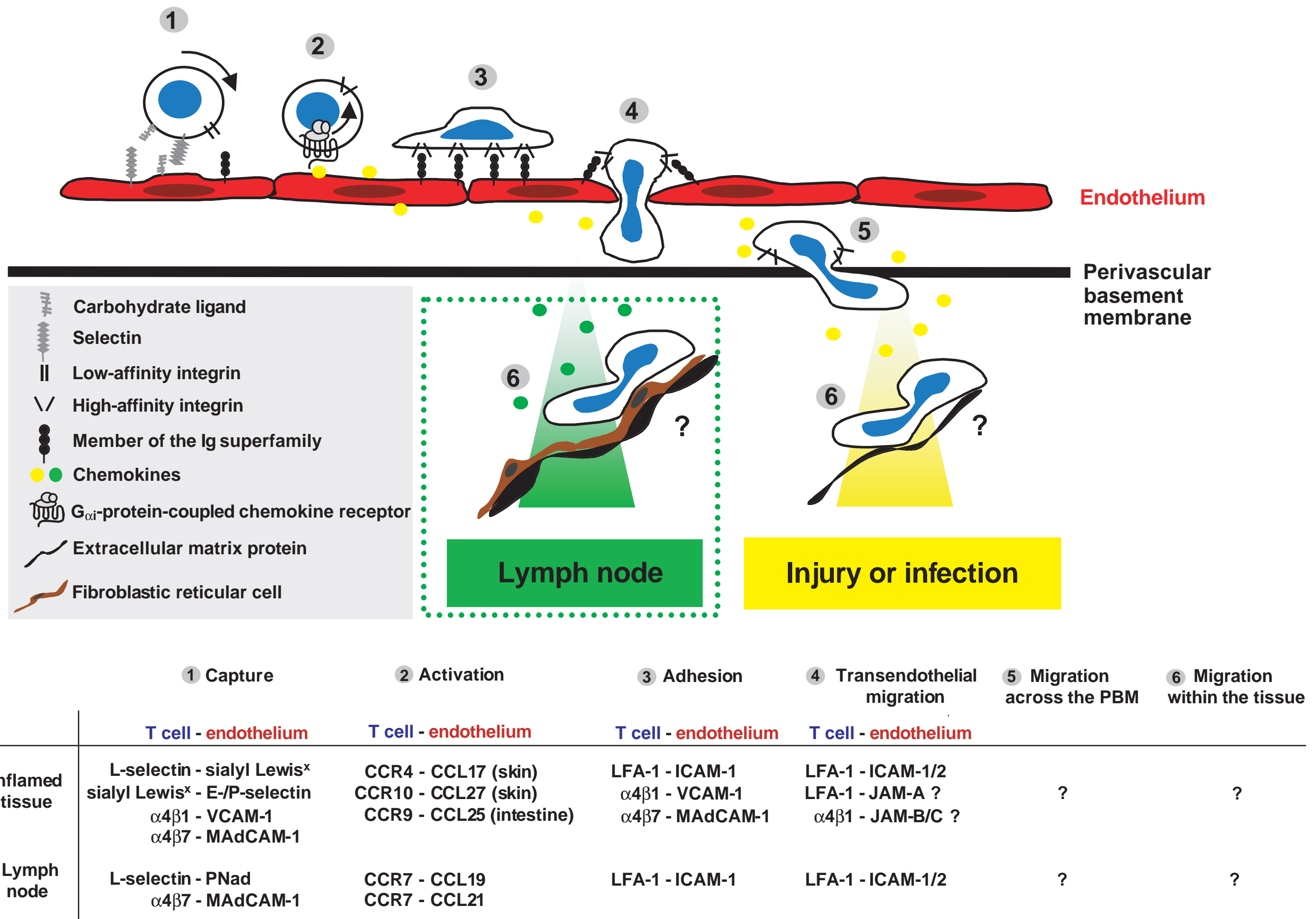
T cells can express at least 12 of the 24 known integrin heterodimers, the expression pattern depending on the subset and maturation state of the cell (reviewed by von Andrian and Mackay, 2000). The four leukocyte-specific β2 integrins (αLβ2, αMβ2, αXβ2, αDβ2) are found on T cells, αLβ2 (leukocyte function-associated antigen-1; LFA-1) being the most abundant and widespread in expression. T cells also express the two β7 integrins (α4β7 and αEβ7) and, in common with many other cell types, the extracellular matrix (ECM)-binding β1 integrins (α1–α6β1). These integrins play a prominent role in T-cell function, for example in the migration of T cells to peripheral lymph nodes and inflammatory sites, and in antigen presentation and cytotoxic killing. Their interactions with ligands must therefore be tightly controlled. Under normal conditions, the integrins are inactive, but exposure to cytokines or chemokines and engagement of other cell-surface receptors results in rapid integrin activation and ligand binding. Here, we detail recent advances in our understanding of the roles of integrins on T cells and their regulation.

Functions of integrins on T cells

Crossing the endothelial barrier

The ability of unchallenged or naive T cells to enter lymph nodes depends on a particular set of adhesion molecules and chemoattractants on the high endothelial venules (HEVs), for which they alone bear receptors (Fig. 1). Entry into inflamed tissue is similar but uses different adhesion molecules and chemoattractants, which attract populations of activated T cells. This highly regulated process allows tissue-specific homing of several T-cell subsets (reviewed by Butcher et al., 1999).

Whether routinely entering a lymph node or responding to an inflammatory signal from injured tissue, a major challenge faced by the T cell is to make the transition from a freely circulating cell to an actively migrating T cell able to cross the vascular endothelium of the blood vessel. Its first encounter with endothelium occurs when L-selectin on T cells binds to its glycosylated ligand on the endothelium or, alternatively, when ligands on the T-cell surface bind E- or P-selectin expressed by stimulated endothelium. The rapid on-off binding of the selectins reduces the velocity of the blood-borne T cell, which subsequently rolls along the vascular wall. Integrins α4β1 and α4β7 can also serve as 'rolling receptors'. This slowing of the T cell allows it to sense agonists, such as chemokines, which are presented on the endothelial surface. Chemokine receptor signalling activates integrins on the T-cell



	1 Capture	2 Activation	3 Adhesion	4 Transendothelial migration	5 Migration across the PBM	6 Migration within the tissue
	T cell - endothelium	T cell - endothelium	T cell - endothelium	T cell - endothelium		
Inflamed tissue	L-selectin - sialyl Lewis^x sialyl Lewis^x - E-/P-selectin α4β1 - VCAM-1 α4β7 - MAdCAM-1	CCR4 - CCL17 (skin) CCR10 - CCL27 (skin) CCR9 - CCL25 (intestine)	LFA-1 - ICAM-1 α4β1 - VCAM-1 α4β7 - MAdCAM-1	LFA-1 - ICAM-1/2 LFA-1 - JAM-A ? α4β1 - JAM-B/C ?	?	?
Lymph node	L-selectin - PNad α4β7 - MAdCAM-1	CCR7 - CCL19 CCR7 - CCL21	LFA-1 - ICAM-1	LFA-1 - ICAM-1/2	?	?

Fig. 1. The multistep model of T-cell migration. T cells migrate from the blood, across the vascular endothelium into infected tissue or into lymph node (LN). The receptors involved in stages 1-6 are indicated in tabular form. The process of crossing the endothelial barrier into infected tissue or LN is similar. However, in LN, T cells crawl within a network of fibroblastic reticular cell-coated cables and, in infected tissues, they migrate along matrices composed chiefly of collagen fibrils. Abbreviations: CCL, chemokine ligand; CCR, chemokine receptor; PBM, perivascular basement membrane; ?, lack of information.

surface through the heterotrimeric G protein $G_{\alpha i}$ and thereby enables the T cell to adhere and then migrate across the vascular wall. The most important integrins during this process are LFA-1, α4β1 and α4β7. Their ligands on the endothelium are members of the immunoglobulin superfamily named intercellular adhesion molecule (ICAM)-1, ICAM-2, vascular cell adhesion molecule (VCAM)-1 and mucosal addressin cell adhesion molecule (MAdCAM)-1. Chemokines such as CXCL12 (SDF-1) switch α4 integrin to a high-avidity (clustered) state resulting in the prompt arrest of T cells on the endothelium within 0.1 seconds (Grabovsky et al., 2000). The fact that α4 integrin is prominently displayed on the microvilli of activated T cells supports the idea that it acts as the capture integrin on the vasculature, whereas LFA-1, which is only expressed on the T-cell body, takes over once the T cell rolls along the endothelium (Berlin et al., 1995; Dunne et al., 2002). LFA-1/ICAM-1, not α4 integrin/VCAM-1, mediates migration of T cells across endothelial cells in vitro (Laschinger and Engelhardt, 2000; Oppenheimer-Marks et al., 1991). This fits with the observations that VCAM-1 is expressed only apically, whereas ICAM-1 is also found laterally on the endothelium (Oppenheimer-Marks et al., 1991). The initial adhesion and spreading of the T cell on endothelium leads to redistribution of VCAM-1 and ICAM-1 into a ‘landing pod’ docking structure that partially envelopes the T cell (Barreiro et al., 2002). Once the T cell begins to migrate using LFA-1, the VCAM-1 docking structure vanishes, but ICAM-1 remains clustered.

The junctional adhesion molecule (JAM)-family members are also involved in transmigration (reviewed by Muller, 2003). JAM-A is a ligand for LFA-1 and is found both apically and at the tight junctions of endothelial cells (Ostermann et al., 2002). An antibody against JAM-A blocks T-cell transmigration, indicating that LFA-1 may swap ligands from the ICAMs to JAM-A as the T cell transmigrates. Furthermore, another member of this family, JAM-B, binds to α4β1 on T cells in a manner dependent upon JAM-C (Cunningham et al., 2002).

Increasing evidence indicates that the function of one type of integrin can be modulated by the ligation of another. Engagement of α4β1, for example, can activate binding of LFA-1 to ICAM-1 (Chan et al., 2000). Similarly, binding of ICAM-1 to LFA-1 alters the function of α4β1 and α5β1 on T

cells (Leitinger and Hogg, 2000; Porter and Hogg, 1997). One situation where such crosstalk might occur is during transendothelial migration, when T cells switch from α4-mediated rolling/adhesion to LFA-1-mediated adhesion/ migration. Unstimulated endothelium constitutively expresses ICAM-1, although at low levels, and ICAM-2. Therefore, circulating T cells are continually exposed to LFA-1 ligands on the vasculature, and their ligand-binding activity needs to be tightly regulated. Control of LFA-1 activity by α4β1 may serve this purpose. However, we know little about cooperation between these two integrins during T-cell recruitment into tissue and in other aspects of immunity.

Migration within tissues

T cells must not only cross the endothelial cells lining the vascular wall, but must also migrate through the perivascular basement membrane (PBM). Because the PBM is rich in integrin ligands such as laminin and collagen type IV, integrins are good candidates for mediating this process. In vivo studies show that neutrophil migration across the endothelium causes α6β1 activation, which the neutrophil uses to migrate through the laminin-containing PBM (Dangerfield et al., 2002). Whether the same applies to T cells is unknown. Once across the PBM, T cells follow a chemotactic gradient along large collagen fibrils to the source of inflammation (reviewed by Dustin and De Fougerolles, 2001) (Fig. 1). Time-lapse video microscopy of T cells migrating in three-dimensional (3D) collagen matrices reveals that the collagen fibres act as 'tramlines' to guide the highly motile T cell through the ECM (Friedl et al., 1998). During this process, the β1 integrins are clustered at both the leading edge and uropod of the T cell; however, blocking the function of the collagen-binding integrins with β1 monoclonal antibodies (mAbs) does not influence T-cell attachment or motility (Friedl et al., 1998). It is therefore possible that integrin-independent mechanisms might mediate T-cell migration through the ECM. For example, the discoidin domain receptor (DDR) family of receptor tyrosine kinases bind and are activated by collagen and have been proposed to mediate migration of leukocytes in 3D collagen matrices (Kamohara et al., 2001). Recently, de Fougerolles et al. demonstrated that mAbs specific for α1β1 and α2β1 integrins efficiently inhibit the inflammatory response in several mouse models (de Fougerolles et al., 2000). This is the first evidence for a role for these collagen-binding integrins in immune responses in tissues. However, this study did not discriminate between inhibitory effects on T-cell proliferation, T-cell adhesion or effector function.

Naive T cells enter the lymph nodes via HEVs and migrate to the edge of the node (the paracortex), where they encounter APCs necessary for the initiation of the primary immune response. In the lymph node cortical area, unlike other tissues, matrix proteins are encased in fibroblastic reticular cells, which form a 'labyrinthine cavity' network free of fibrillar matrix, through which the T cells move (Kaldjian et al., 2001) (Fig. 1). By contrast, soluble factors, such as chemokines, are transported within these 'hollow tubes' through this cavity to the surface of the HEV, where they serve as chemoattractants for T cells (Gretz et al., 2000). The T cells then exit the lymph node via lymphatic vessels in the medulla. Much is known about T-cell entry into the nodes, but little about whether integrins are involved in their movement within the node compartments. It would be of interest to know whether integrins aid the T cell in binding to the fibroblastic reticular cells, migrating to different areas of the node, or exiting into the lymphatic circulation. Lo et al. showed recently that B cells use LFA-1 and α4β1 to enter the ICAM-1/VCAM-1-rich marginal zone of the spleen, and only a combination of antibodies directed against both integrins blocked recruitment of these cells (Lo et al., 2003). Whether these integrins have a similar role in guiding T cells through lymph nodes remains to be investigated.

LFA-1 in the immunological synapse

When a T cell engages an APC, such as a dendritic cell, key receptors at the cell-cell contact reorganize to form the 'immunological synapse' (IS) on the surface of the engaged T cell (reviewed by Bromley et al., 2001; Cannon and Burkhardt, 2002). A major feature of the mature IS is the concentration of TCRs at its centre surrounded by a ring of LFA-1 (Monks et al., 1998). In vitro, the APC can be mimicked by a lipid bilayer containing peptide-bound major histocompatibility complex (MHC) class I/II molecules (the TCR ligand) and ICAM-1, and this is sufficient for IS formation, highlighting the essential role of LFA-1 in the process (Grakoui et al., 1999). This model system has revealed that the formation of the IS follows a series of dynamic molecular events. Initially, a central zone of LFA-1 with a peripheral ring of TCRs forms at the contact site, but within minutes this distribution is reversed. The central area becomes occupied by the TCRs, which are associated with the protein kinases PKCθ, Fyn and Lck, whereas LFA-1 becomes clustered as a peripheral ring colocalized with the cytoskeletal protein talin (Grakoui et al., 1999). During this process, the microtubule-organizing centre (MTOC) reorients towards the IS. In T-cell–APC couples formed from Vav-1-deficient T cells, there is a lack of LFA-1 function and the MTOC fails to polarize towards the IS (Ardouin et al., 2003). LFA-1 might thus control MTOC orientation and cell polarization.

Initially, the formation of the IS, which is stable for >1 hour, seemed to be essential for a T-cell-driven immune response (reviewed by Bromley et al., 2001; Cannon and Burkhardt, 2002). More recently, Friedl and colleagues have shown T cells crawling over the surface of dendritic cells, seeking their specific MHC-peptide complex (Gunzer et al., 2000). These interactions are more short-lived, with average encounters lasting 6-12 minutes. The relevance of the stable IS for T-cell programming has also been questioned following the observation that TCR-mediated signalling through the tyrosine kinases Lck and ZAP-70 precedes formation of the mature IS (Lee et al., 2002). This debate has led to speculation that a long-lived synapse might function beyond immediate T-cell stimulation. It could serve as a discrete zone for polarized secretion, endocytosis or recycling of various receptors, such as the TCR (reviewed by Trautmann and Valitutti, 2003).

Integrins and lipid rafts

Another feature of integrin behaviour is that, upon activation, they can be found in lipid rafts. These specialized membrane microdomains are enriched in cholesterol and sphingolipids and serve as platforms for adaptor and signalling molecules in

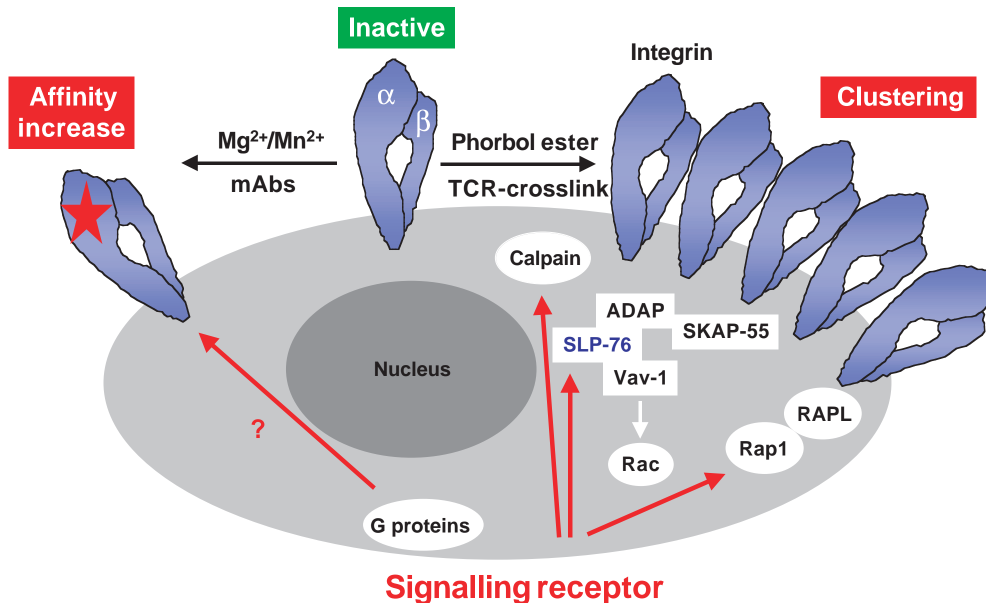


Fig. 2. Integrin activation. An integrin achieves higher affinity for ligand through conformational change or gains strength of binding through clustering (avidity). These two states can be accomplished experimentally through binding of Mg^{2+}, Mn^{2+}, activating mAbs or treatment with phorbol ester or crosslinking TCR. Gene modification of ADAP, SKAP-55 or Vav-1 (complexed with SLP-76; blue) demonstrates their role in integrin clustering. Calpain, GTPases Rac and Rap1, and the Rap1-binding protein RAPL also participate in integrin clustering. Signalling through chemokine receptors to pertussis-toxin-sensitive G proteins can cause a transient increase in integrin affinity. See text for references.

many cell types, including T cells (reviewed by Alonso and Millan, 2001; Cherukuri et al., 2001; Simons and Toomre, 2000). T cells contain two major classes of raft defined by their glycosphingolipid content. Ganglioside GM3-containing rafts localize to the leading edge, whereas ganglioside GM1-containing rafts are associated with the uropod (or trailing edge) of polarized T cells (Gomez-Mouton et al., 2001). Active LFA-1 and α4β1 colocalize with GM1-enriched rafts in Jurkat T cells (Leitinger and Hogg, 2002), and β1 integrins associate with the lipid raft fraction upon GM1 aggregation in a lymphocytic cell line (Mitchell et al., 2002). Additionally, LFA-1-mediated adhesion of mouse thymocytes (Krauss and Altevogt, 1999), and human T-cell adhesion through LFA-1 and α4β1 (Leitinger and Hogg, 2002), is abolished when lipid rafts are disrupted by the extraction of cholesterol with methyl-β-cyclodextrin. These data suggest that activation of integrins results in their localization to a lipid raft compartment and that this association is essential for integrin-mediated T-cell adhesion.

Alon and colleagues have shown that, in contrast, cholesterol extraction has no effect on α4β1-, α4β7- and LFA-1-mediated adhesion of fresh peripheral blood T cells to VCAM-1, MAdCAM-1 and ICAM-1, respectively (Shamri et al., 2002). Rapid α4β1-mediated capture of T cells on endothelium in response to the chemokine SDF-1 under flow conditions is sensitive to cholesterol extraction. However, it is the SDF-1 chemokine receptor, and not α4 integrin, that is localized in the lipid raft microdomain. Furthermore, although LFA-1 is found in the raft compartment under these conditions, its ability to bind to ICAM-1 is insensitive to raft disruption. The discrepancy between these findings and the reports mentioned above could be due to the state of activation of the T cells under investigation. Peripheral blood T cells have low levels of GM1 expression compared with T-cell lines (K.G., unpublished). At low levels of lipid raft coalescence, ligand-bound integrin might preferentially engage raft-independent signalling cascades.

There is much to learn about how integrins are recruited and retained within the lipid raft compartment. Neither subunit of the integrin heterodimer is modified by palmitoylation, a characteristic of many raft transmembrane proteins. It is conceivable that raft localization is dependent on the formation of complexes of integrins with other membrane proteins. For example, tetraspanin proteins such as CD81 and CD82 can be found in lipid rafts (Claas et al., 2001). Overexpression of CD82 and binding of antibodies to CD81 potentiate LFA-1-mediated adhesion (Shibagaki et al., 1999; VanCompernolle et al., 2001), but whether these tetraspanins are associated with LFA-1 within lipid rafts is unknown. In addition, we know little about the heterogeneity of receptors and signalling molecules in rafts. Which raft subsets are associated with integrins might determine the availability of signalling molecules and hence the signalling output.

Regulation of integrins on T cells

Activation of integrins: insight into inside-out signalling

As already mentioned, the ligand-binding activity of LFA-1 needs to be tightly regulated. However, even when activated, LFA-1 has a relatively low affinity for its ligands (K_d of ~130 nM), which is appropriate for the rapid making and breaking of adhesions (Woska et al., 1996). Integrin activation results from either clustering of integrins on the cell surface, or an increase in the affinity for ligand induced by conformational change (Fig. 2). Both are tightly regulated by a complex interplay of cation binding, signalling events and associations with the cytoskeleton and accessory molecules (reviewed by Stewart and Hogg, 1996; van Kooyk and Figdor, 2000).

Ligation of cell-surface receptors, such as the TCR or chemokine receptors, generates intracellular signals that increase LFA-1-mediated cell adhesion. This is termed inside-out activation. Addition of the divalent cations Mn^{2+} or Mg^{2+} or activating antibodies that bind to the extracellular portion of LFA-1 cause conformational changes that also activate LFA-1. This is termed outside-in activation. In vitro, outside-in activation increases the affinity of LFA-1 for its ligands,

whereas inside-out activation induces an easily detectable increase in its lateral mobility and clustering (Kucik et al., 1996; Stewart et al., 1996; Stewart et al., 1998; van Kooyk et al., 1994). Whether inside-out activation leads to a change in integrin affinity, clustering, or both has been debated (Bazzoni and Hemler, 1998), but there is now evidence for the latter situation.

Integrin affinity modulation

Signals that increase the affinity of integrins on leukocytes have been difficult to identify. Recently, Constantin and colleagues showed that μM levels of chemokine cause a rapid and transient increase in the affinity of LFA-1 on mouse thymocytes and a longer-lasting increase in clustering (Constantin et al., 2000). Similarly, α4β1 affinity rapidly increases on monocytes following chemokine stimulation (Chan et al., 2001). These studies suggest that inside-out signalling through receptors on the T cell can lead to an increase in the affinity of integrins, which has previously been undetected owing to its transience.

Other evidence suggests that integrins exist in an equilibrium of low- and high-affinity forms on the T-cell membrane and that the maturation and activation state of the T cell influences the balance between these forms. Recent data point to a role for the Src-family kinase Lck in maintaining high-affinity α4β1 on circulating T cells and reveal a requirement for this high-affinity integrin in the rapid arrest of T cells on stimulated endothelium in vitro (Feigelson et al., 2000). Another possibility is that an increase in integrin affinity results from a conformational change by 'induced fit', following low-affinity interactions with ligand (reviewed by Hogg et al., 2002).

Integrin clustering

Several proteins influence integrin clustering. Inhibitors of the Ca^{2+}-dependent protease calpain inhibit clustering and adhesion of LFA-1 on T cells following TCR signalling, phorbol ester activation or increases in intracellular Ca^{2+} levels (Stewart et al., 1998) (Fig. 2). This has led to the hypothesis that calpain is required for the release of integrins from the cytoskeleton, permitting their lateral mobility in the membrane and subsequent clustering. The signalling pathways activating calpain and the identity of its downstream targets are still being sought. Although the cytoskeletal protein talin is a target in some cells (Sampath et al., 1998), this has not been confirmed in T cells (A.M., unpublished).

Studies of transgenic and knockout mice models have identified the guanine nucleotide exchange factor (GEF) Vav-1 (Ardouin et al., 2003; Krawczyk et al., 2002), the adaptor proteins ADAP (also known as SLAP-130 or Fyb) (Griffiths et al., 2001; Peterson et al., 2001) and SKAP-55 (Wang et al., 2003), and the small GTPase Rap1 (Katagiri et al., 2000; Sebzda et al., 2002) as key players in the pathway between TCR activation and LFA-1 clustering. Following phosphorylation, SKAP-55 binds to ADAP, and ADAP binds to the adaptor protein SLP-76, which is also found in complex with Vav-1. Vav-1 is a GEF for the GTPase Rac, and expression of active Rac in T cells causes LFA-1-mediated spreading and clustering (D'Souza-Schorey et al., 1998). Alternatively, Vav-1 can promote T-cell spreading by a GEF-independent mechanism (del Pozo et al., 2003). The mechanism by which Rap1 induces LFA-1 clustering and adhesion was unknown until very recently. RAPL has now been identified as a protein that binds to GTP-bound Rap1 and is required for Rap1-mediated redistribution and clustering of LFA-1 upon TCR or chemokine stimulation (Katagiri et al., 2003).

Signalling through the TCR can cause lateral interactions of integrins with other transmembrane proteins. For example, β1 integrin interacts with CD98 (Miyamoto et al., 2003) and β2 integrin interacts with DNAM-1 (Shibuya et al., 1999), CD81 and CD82. Most importantly, the association of integrins with these transmembrane proteins has been shown to enhance integrin-mediated adhesion (Shibagaki et al., 1999; VanCompernolle et al., 2001).

The role of kinases

We have known for many years that activation of PKC by phorbol esters triggers LFA-1-mediated adhesion. Overexpression of the conventional PKC isoforms (PKCα, PKCβI, PKCβII and PKCδ) activates LFA-1 (Katagiri et al., 2000). The receptor for activated PKC (Rack1) associates with the integrin β2 subunit upon phorbol ester stimulation of the JY lymphoblastoid cell line (Liliental and Chang, 1998), and this interaction can recruit activated PKC to sites of cell adhesion and regulate the interaction between LFA-1 and the cytoskeleton. PKC phosphorylates several residues in the β2 integrin subunit, and phosphorylation of Thr758 allows binding of the scaffolding protein 14-3-3 to its cytoplasmic tail (Fagerholm et al., 2002). This family of proteins binds to phospho-serine and -threonine motifs, allowing the sequestration of signalling molecules.

Interestingly, although chemokine-induced LFA-1 clustering requires phosphoinositide 3-kinase (PI3-kinase) (Constantin et al., 2000), a catalytically inactive mutant of the haematopoietic-cell-specific p100 δ subunit of PI3-kinase does not affect TCR-induced adhesion of mouse T cells to the LFA-1 ligand ICAM-1 or the α4 integrin ligand fibronectin (Okkenhaug et al., 2002). This suggests that the TCR and chemokine receptors might activate T-cell integrins by different routes. Finally, the Tec-family tyrosine kinase Itk, which is upstream of PI3-kinase and downstream of phospholipase Cγ (PLCγ) and Src kinase (reviewed by Takesono et al., 2002), regulates β1-integrin-mediated adhesion in T cells when activated through the TCR (Woods et al., 2001).

Human integrin disorders

Valuable information about integrin activation has come from the study of patients with genetic lesions causing faulty integrin function. Two types of integrin dysfunction affect cells of haematopoietic origin (Hogg and Bates, 2000). The most frequent one, Glanzmann's thrombasthenia, involves the platelet integrin αIIbβ3 and gives rise to a bleeding tendency. The second is the β2 integrin disorder termed leukocyte adhesion deficiency 1 (LAD-1), which causes severe bacterial infections and other immune deficiencies. Recently, McDowall et al., identified a patient whose platelets and leukocytes express normal levels of β1, β2 and β3 integrins but exhibit dysfunction of all three classes of integrin owing to defective

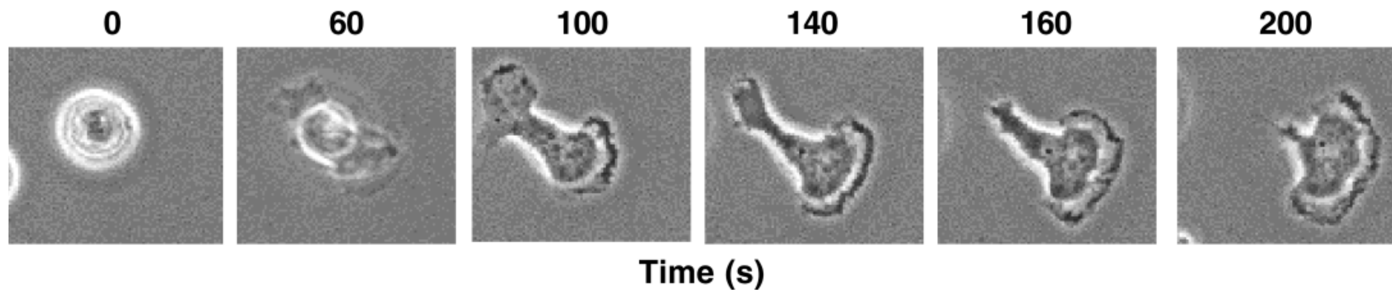


Fig. 3. LFA-1-mediated migration of T cells on ICAM-1. Time-lapse video microscopy (×40 magnification) of a primary human T cell in contact with immobilized ICAM-1. The time in seconds after the first contact with ICAM-1 is depicted in each frame: initial contact (time=0), T-cell polarization (time=60-160 seconds) and migration (time=200 seconds) (Smith et al., 2003). Data reproduced with kind permission of Andrew Smith, Cancer Research, UK.

inside-out signalling pathways (McDowall et al., 2003). The defect is associated with constitutive integrin clustering. This might seem counterintuitive, but it might be that it is the dynamics of clustering necessary for adhesion that is faulty. Alternatively, the clusters might be incorrectly distributed on the cell membrane. Interestingly, three other patients have similar disease characteristics, but with some distinctive aspects. Thus, they might possess different lesions in a common pathway that is key to integrin activation (Alon et al., 2003; Harris et al., 2001; Kuijpers et al., 1997). Identification of the lesions in these patients should provide valuable insight into the signalling that controls the activity of at least the β1, β2 and β3 integrins on haematopoietic cells.

Signalling through integrins: outside-in signalling

Since integrins cooperate with other receptors, such as the TCR, it has been difficult to determine whether they can signal directly (outside-in signalling) or whether they merely perform an adhesive function that aids signalling through other receptors. Studies in which integrins are activated directly by specific antibodies or the divalent cations Mg^{2+} and Mn^{2+} in the presence of ligand have now established that T-cell integrins can indeed signal, and such approaches are helping to dissect downstream signalling.

Signalling events that follow direct LFA-1 engagement include tyrosine phosphorylation of PLCγ1 (Kanner et al., 1993) and activation of the tyrosine kinases ZAP-70 (Soede et al., 1999), Pyk-2 and FAK (Rodriguez-Fernandez et al., 1999). Clustering of β1 integrins on Jurkat T cells causes the PKC-dependent serine phosphorylation of the protein kinase Lck, leading to the tyrosine phosphorylation of Shc (Niu et al., 2003).

Cytoskeletal rearrangement and cell migration

It is becoming evident that signalling through LFA-1 leads to T-cell migration. The binding of Mg^{2+}-stimulated LFA-1 to ICAM-1 causes T-cell polarization within 1-2 minutes, leading to cell motility within 2-3 minutes (Smith et al., 2003) (Fig. 3). The cells then migrate randomly at 10-15 μm/minute. Recently, intravital two-photon microscopy has provided a bird's eye view of the movement of T cells in the inguinal lymph node in vivo. Here, the naive $CD4^+$ T cells migrate randomly at ~11 μm/minute (Miller et al., 2003). This speed contrasts with the 10 μm/hour of a typical fibroblast (Abercrombie, 1961).

The F-actin cytoskeleton dynamically alters as the T-cell migrates on ICAM-1. Each cell possesses only 3.4 pg of cortical F-actin (Phatak et al., 1988), which is increased by ~30% following LFA-1 engagement with ICAM-1 (Porter et al., 2002). Much of this new F-actin is located at the leading edge of the migrating T cell, supporting the lamellipodial extensions. Cell migration also depends on dynamic changes to the actomyosin cytoskeleton regulated by myosin light chain (MLC) kinase (MLCK) and Rho kinase (ROCK), both of which phosphorylate MLC, thereby activating myosin. These kinases are spatially segregated in the T cell: MLCK operates at the leading edge to control T-cell adhesion and lamellipodial extension, whereas ROCK is involved in the retraction of the trailing edge (see below) (Smith et al., 2003) (Fig. 4). Further investigation is needed to understand how LFA-1 controls the activity of these kinases.

LFA-1 also interacts with the microtubule network. LFA-1 engagement promotes translocation of Pyk-2 (Rodriguez-Fernandez et al., 1999), PKCβI and PKCδ (Volkov et al., 1998) to the MTOC of the migrating T cell. PKCβI also colocalizes with microtubules, and LFA-1 has been isolated as part of a PKCβI–tubulin-rich complex in T cells. T cells lacking PKCβI cannot migrate on ICAM-1, which indicates that it is required for LFA-1-activated T-cell motility (Volkov et al., 1998; Volkov et al., 2001). Interestingly, it plays no part in the F-actin-dependent lamellipodial extensions.

As well as migrating randomly, T cells also migrate under the direction of chemotactic stimulants such as chemokines (del Pozo et al., 1995). Sanchez-Madrid and colleagues have detailed many changes that occur under these conditions (reviewed by Sanchez-Madrid and del Pozo, 1999; Serrador et al., 1999). Following exposure to a chemokine, T cells polarize and relocate key membrane receptors, particularly associated with adhesion/migration, either to the leading edge or to the uropod compartments of the T cell. Chemokine receptors are found at the front of the T cell (Nieto et al., 1997), whereas ICAM-1/3, CD43 and CD44 are concentrated in the uropod. The cytoskeletal-linking ERM proteins (ezrin, radixin and moesin) are involved in this redistribution because, for example, ICAM-3 colocalizes with moesin (Serrador et al., 2002).

Although similar studies have not been carried out for randomly migrating T cells, receptor compartmentalization

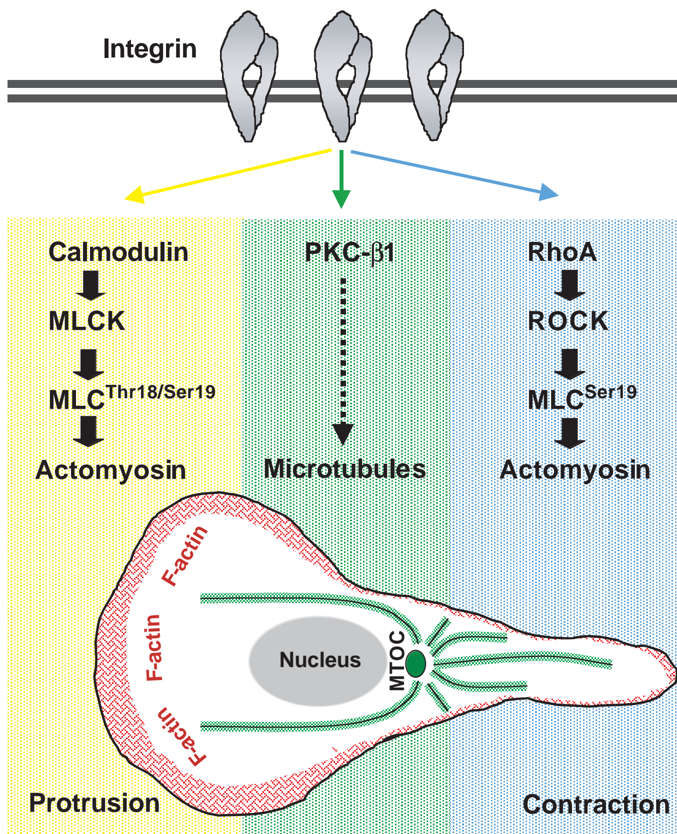


Fig. 4. Kinases involved in LFA-1-mediated migration. Signalling through LFA-1 results in major changes in the T-cell morphology leading to migration. There are two compartments of myosin motor activity. At the leading edge, T-cell attachment and lamellipodial extension is dependent upon myosin, which is activated by MLCK. At the rear of the cell, myosin activated by RhoA and ROCK results in detachment of the trailing edge. The association of PKCβI with microtubules promotes T-cell migration (see text for details and references).

might well be similar. In vitro random migration on ICAM-1 is initiated through LFA-1 activation, but what controls the 'random walk' of in vivo migrating T cells is not known. It would be of interest to investigate the positioning of the chemokine receptors, although there is a lack of evidence so far that chemokines control in vitro random migration (A. Smith, personal communication).

GTPases, adhesion and migration of T cells

In fibroblast models, the Rho-family GTPases, RhoA, Rac and Cdc42 have well-understood roles in stress fibre and focal adhesion formation, lamellipodial extension, and filopodial extensions, respectively (reviewed by Hall, 1998; Ridley, 2001). However, T cells do not form easily identifiable 'classical' adhesion structures such as focal adhesions, focal contacts or actin stress fibres. Thus, are integrin signals interpreted differently in T cells? The Rho-family GTPases are expressed by T cells, and some evidence suggests that they operate in ways similar to those in the fibroblast. For example, an active Rac mutant causes $\alpha4\beta1$- and $\alpha5\beta1$-mediated spreading of Jurkat T cells on fibronectin by promoting integrin clustering (D'Souza-Schorey et al., 1998). By contrast, other studies show that activating mutants of Rho, Rac and Cdc42 block polarization of T-cell lines on fibronectin (del Pozo et al., 1999). These conflicting results might reflect the fact that individual Rho GTPases can both antagonize and activate other family members. Therefore, the outcome of transfection of a single mutant GTPase might depend on the level of its expression as well as that of endogenous GTPases.

Interfering with RhoA activity using C3 exoenzyme prevents detachment of the trailing edge and dramatically reduces the rate of migration of T cells (Smith et al., 2003; Woodside et al., 2003), monocytes (Worthylake et al., 2001) and eosinophils (Albas et al., 2001). This requirement for RhoA function for cell detachment appears to be a common feature of the migrating leukocyte; by contrast, in non-haematopoietic cells, inhibition of RhoA can facilitate rear release by disrupting focal adhesions (Ridley, 2001).

Inhibition of the RhoA effector ROCK (or Rho kinase) in T cells also results in a failure of the trailing edge to detach (Smith et al., 2003). Although ROCK has many potential downstream effectors (Riento and Ridley, 2003), MLC is a key target in the migrating T cell. Inhibition of ROCK using the inhibitor Y-27632 blocks phosphorylation of Ser19 of MLC (Smith et al., 2003), which is required for the contraction of actomyosin fibrils. RhoA/ROCK might thus function at the rear of the cell to disrupt adhesions mechanically by stimulating actomyosin contraction. The enhanced adhesion of T cells that occurs upon ROCK inhibition may also be explained by the observation that inhibition of RhoA or ROCK in HSB-2 T cells promotes integrin clustering, which suggests that RhoA/ROCK has a role in unclustering integrins (Rodriguez-Fernandez et al., 2001).

Attention has recently focused on the GTPase Rap1, after Kinashi and colleagues provided convincing evidence of its importance in T-cell migration. In T cells, Rap1 is activated by chemokines (Shimonaka et al., 2003), as well as by TCR engagement when it localizes to the IS (Katagiri et al., 2000). Expression of a dominant-active form of Rap1 (Rap1V12) enhances LFA-1 clustering and adhesion (Katagiri et al., 2000; Sebzda et al., 2002). Rap1V12 expression also induces motility on ICAM-1 and VCAM-1 at speeds of ~25 μm/minute, which is similar the speed of T-cell migration induced by chemokine stimulation (Shimonaka et al., 2003). The pathway whereby Rap1 causes LFA-1 activation and clustering involves a newly identified protein, RAPL (Katagiri et al., 2003). After TCR or chemokine stimulation, RAPL binds to GTP-bound Rap1 and coprecipitates with LFA-1. This association of RAPL with LFA-1, although perhaps not direct, is required for Rap1-induced redistribution of LFA-1 and is disrupted by mutation of lysines 1097 and 1099 in the LFA-1 α subunit cytoplasmic tail (Katagiri et al., 2003; Tohyama et al., 2003).

Adaptor and cytoskeletal proteins

More than 20 different integrin-binding molecules have been identified (Liu et al., 2000), but the number of adaptor and cytoskeletal proteins proposed to interact with the cytoplasmic tails of leukocyte integrins is far fewer. $\beta1$ integrins from Jurkat T-cell lysates coprecipitate with filamin (Loo et al., 1998), and $\beta2$ integrins from leukocytes associate with filamin, α-actinin and talin (Sampath et al., 1998; Sharma et al., 1995; Valmu et

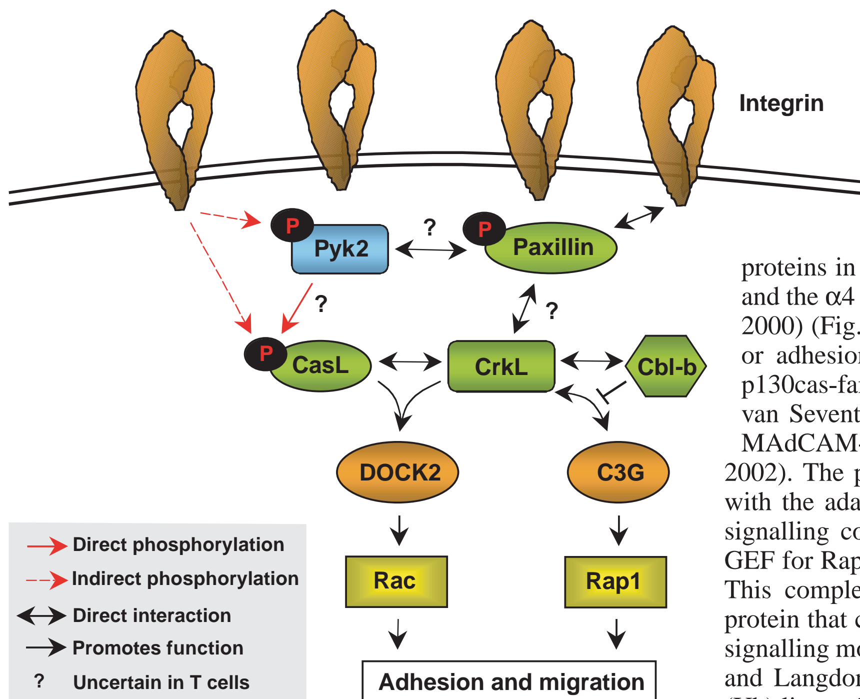


Fig. 5. Signalling through integrins: the paxillin/CasL/CrkL pathway. The CasL/CrkL adaptor complex is formed by signalling through α4β1, α4β7 and LFA-1 on T cells. The figure generalizes the interactions following integrin activation, not all of which have been demonstrated for each T-cell integrin (see text for details).

al., 1999). In addition, talin colocalizes with LFA-1 at the IS (Monks et al., 1998).

The adaptor protein cytohesin-1 is of interest because it binds selectively to the β2 subunit of LFA-1 (Kolanus et al., 1996). Cytohesin-1 is a cytoplasmic protein containing an N-terminal coiled-coil domain, a Sec-7 domain and a C-terminal pleckstrin homology (PH) domain, the last two domains participating in LFA-1 function. Over-expression of cytohesin-1 or its Sec-7 domain enhances adhesion of Jurkat T cells to ICAM-1 (Kolanus et al., 1996), as well as chemokine-stimulated cell capture on and migration across endothelium (Weber et al., 2001). Cytohesin-1 associates with the cell membrane through its PH domain. Over-expression of the recombinant PH domain blocks LFA-1 function, which suggests that cytohesin-1 must associate with the membrane to modulate LFA-1 activity (Nagel et al., 1998). Furthermore, the protein CYTIP, which binds to cytohesin-1, removes it from the membrane and thereby downregulates adhesion of T cells to ICAM-1 (Boehm et al., 2003). The cytohesin-1 Sec-7 domain acts as a GEF for the ARF family of vesicle trafficking GTPases and, through ARF-6, causes spreading of Jurkat T cells (Weber et al., 2001). Additionally, cytohesin-1, when phosphorylated by PKCδ, associates with actin, and this could provide the link between LFA-1 and the cytoskeleton (Dierks et al., 2001).

Paxillin is a multidomain adaptor protein central to signalling during adhesion of several cell types (reviewed by Turner, 2000). It binds directly to the α4 integrin tail in Jurkat T cells and promotes activation of kinases FAK and Pyk2, leading to decreased cell spreading and increased migration (Liu et al., 1999; Rose et al., 2003). In T cells, paxillin localizes to areas of LFA-1 and α4β1 contact with ligand, as well as at the MTOC (Herreros et al., 2000). Pyk2 also localizes to the MTOC upon LFA-1 engagement (Rodriguez-Fernandez et al., 1999), and by interacting with paxillin at this location may promote further signalling and cytoskeletal reorganization.

There is increasing evidence for a role for the p130cas and Crk families of adaptor proteins in adhesion and migration mediated by both LFA-1 and the α4 integrins (reviewed by Feller, 2001; O'Neill et al., 2000) (Fig. 5). In T cells, crosslinking α4β1 with antibodies or adhesion to fibronectin induces phosphorylation of the p130cas-family member CasL/HEF-1 (Ohashi et al., 1999; van Seventer et al., 2001). In addition, binding of α4β7 to MAdCAM-1 results in CasL phosphorylation (Murata et al., 2002). The phosphorylation of CasL promotes its interaction with the adaptor CrkL. This pair could function as a central signalling complex in T cells. CrkL associates with C3G, a GEF for Rap1 that is involved in T-cell migration (see above). This complex is negatively regulated by Cbl-b, an adaptor protein that can associate with CrkL as well as various crucial signalling molecules (ZAP-70, PI3-kinase) (reviewed by Thien and Langdon, 2001). Cbl-b can function as an E3 ubiquitin (Ub) ligase through binding to Ub-loaded conjugation enzyme E2 and promoting the transfer of Ub to the target substrate. Cbl-b induces ubiquitylation of CrkL, preventing its interaction with C3G and thus reducing activation of Rap1 (Zhang et al., 2003). T cells from Cbl-b$^{-/-}$ mice show elevated levels of Crk-C3G complex formation, increased Rap1 activity, LFA-1 clustering and adhesion to ICAM-1 (Zhang et al., 2003).

The Crk-Cas complex also associates with the DOCK180 family of proteins, which activate Rho-family GTPases in several cell types despite lacking classical GEF domains (reviewed by Reif and Cyster, 2002). Recently, Nishihara et al. observed that association of CrkL and DOCK2 increases Rac activity in T-cell lines (Nishihara et al., 2002). DOCK2$^{-/-}$ mice show defective homing in response to chemokines CXCL12, CCL19 and CCL21, implicating DOCK2 in T-cell migration (Fukui et al., 2001).

Concluding remarks

We now recognize that T cells require morphological flexibility and rapid migratory behaviour to fulfil their role as immunological cells and that integrins have a key function in controlling these activities. Despite much work, we do not yet fully understand how the activity of integrins on T cells is regulated. Furthermore, we have yet to discover the full signalling potential of integrins on T cells and to understand what information they uniquely contribute when cooperating with other receptors, for example, at the IS. Unlike other studied cell types, T cells do not make easily identifiable focal adhesions or focal complexes, and a challenge is to characterize the form of integrin attachments that enable the T cell to migrate. It will be interesting to compare such information with the wealth of knowledge available for cells such as fibroblasts, which spend their lives in a more sedentary fashion, adherent for much longer time periods.

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