Immunology
The Journal of cells, molecules, systems and technologies

British Society for immunology

IMMUNOLOGY REVIEW ARTICLE

Integrin signalling and function in immune cells

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doi:10.1111/j.1365-2567.2011.03549.x
Received 2 November 2011; revised 7 December 2011; accepted 19 December 2011.
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Summary

Integrins not only mediate cell–cell and cell–extracellular matrix adhesion, but also affect the multitude of signal transduction cascades in control of cell survival, proliferation, differentiation and organ development. Mutations in integrins or the major effectors of integrin signalling pathways cause defective organ development, immunodeficiency, cancer or autoimmune disease. Understanding of the signalling events that drive integrin activation and signalling is therefore crucial to uncover the molecular mechanisms of these diseases. This review discusses the key signalling complexes regulating integrin activation and function in both 'inside-out' and 'outside-in' pathways in T lymphocytes, including kinases, SLP-76, VAV1, ADAP, SKAP-55, RapL, RIAM, Rap1, Talin and Kindlin.

Keywords: adaptor proteins; cytoskeleton; inside-out signalling; integrin activation; outside-in signalling

Integrin families and functions

Integrins are transmembrane adhesion receptors that mediate cell–cell and cell–extracellular matrix adhesion and also induce bidirectional signalling across the cell membrane to regulate cell proliferation, activation, migration and homeostasis.[1] Each integrin contains one α subunit and one β subunit. So far, eighteen α subunits and eight β subunits have been characterized that form 24 different integrins in vertebrates. Studies from gene knockout mice lacking different α and β subunits have indicated that various integrins play crucial roles during development of different organs. α5 knockout mice show vascular defects, and α4 knockout mice have impaired cardiac development.[2,3] α3 knockout mice are perinatally lethal with marked abnormalities in lung development and α6 knockout mice develop severe skin blistering.[4,5] Except for their crucial role in organ development, integrins participate in the process of wound healing, cancer, immune responses against infection and autoimmune diseases. At least 12 integrins are expressed in various types of leucocytes and platelets (Table 1).[6] Accumulation of evidence from human and mouse models has shown that defects in integrin expression or activation in these immune cells result in serious immunodeficiency or autoimmune diseases. Mice with null mutations of the α_L or β_2 subunit show phenotypes similar to patients with leucocyte adhesion deficiency I, including spontaneous infections, impaired leucocyte adhesion and migration to the inflamed and infected skin.[7] In this context, integrins have served as potential therapeutic targets for diseases, such as blocking antibodies to very late antigen-4 ($\alpha_4\beta_1$) (i.e. natalizumab) and leucocyte function-associated antigen-1 (LFA-1; $\alpha_L\beta_2$; or CD11a CD18) (i.e. efalizumab) in the treatment of multiple sclerosis and psoriasis, respectively.[8,9] In the past decades, numerous studies have emerged to propose models of integrin activation and have identified key effectors that could regulate integrin activation. These studies might provide new target molecules to treat patients with these immune cell-based disorders.

Models of integrin activation

Integrin conformational changes are thought to convert integrin affinity from low or intermediate levels to high levels. As a transmembrane receptor, the extracellular parts of α and β subunits form a ligand-binding headpiece and the transmembrane parts are followed by short cytoplasmic tails. In a resting state, the ligand-binding headpiece of an integrin is bent and close to the cell membrane, whereas the cytoplasmic tails are close together to form a conformation with low affinity. When the cytoskeletal protein talin binds to the tail of the β subunit, it is sufficient to expose the epitopes in the stalk regions and generate a conformation with intermediate affinity. Once the

Table 1. Distribution and functions of major integrins in leucocytes

Integrin names	Ligands	Expression patterns	Major functions
$\alpha_L\beta_2$ (LFA-1, CD11a/CD18)	ICAM-1-5, TLN, Type I collagen	T cell, B cell, monocyte/macrophage, NK cell, DC, neutrophil, eosinophil	Migration of T cell, monocyte, neutrophil and eosinophil, activation and adhesion of T cell, DC and B cell, cytotoxicity of CTL and NK cell[85–87]
$\alpha_M\beta_2$ (Mac-1, CR3, CD11b/CD18)	ICAM-1, ICAM-2, ICAM-4, iC3b, fibrinogen, factor X, heparin, laminin, LPS, zymosan, oligodeoxynucleotide, collagen, elastase	Monocyte/macrophage, DC, neutrophil, eosinophil, basophil, NK cell	Adhesion, activation and phagocytosis of macro phage, monocyte, neutrophil, eosinophil, basophil, DC and NK cell[79,88]
$\alpha_X\beta_2$ (p150/95, CR4, CD11c/CD18)	C3bi, fibrinogen, collagen, CD23 heparin, LPS denatured protein	Monocyte/macrophage, neutrophil, NK cell	Adhesion and phagocytosis of monocyte/macrophage and neutrophil, adhesion of NK cell[6,88]
$\alpha_D\beta_2$ (CD11d/CD18)	ICAM-3, VCAM-1	Macrophage, eosinophil, T cell, NK cell	Adhesion and migration of macrophage and eosinophil, adhesion of T cell[89]
$\alpha_4\beta_1$ (VLA-4, CD49d/CD29)	VCAM-1, MAdCAM-1, fibrinogen, fibronectin, thrombospondin, pro-vWF, osteopontin, chondroitin	T cell, B cell, DC, monocyte/macrophage, NK cell, neutrophil, eosinophil, basophil	Migration and development of T cell and B cell, migration of monocyte/macrophage, eosinophil, and DC cell, adhesion of NK cell and neutrophil[90–92]
$\alpha_4\beta_7$ (LPAM-1, CD49d/CD-)	MAdCAM-1, VCAM-1, fibrinogen, fibronectin, osteopontin	T cell, B cell, monocyte/macrophage, NK cell, DC, eosinophil, basophil	Migration and development of T cell and B cell, migration of monocyte, macrophage, DC, eosinophil and NK cell[90,93]
$\alpha_E\beta_7$ (HML-1, CD103/CD-)	E-cadherin	T cell, Treg, NK cell, DC, macrophage	Adhesion and activation of T cell and DC, cytotoxicity of T cell, suppressive function of Treg, recruitment of NK cell and macrophage[94–96]
$\alpha_V\beta_3$ (CD51/CD61)	Vitronectin, ICAM-1, VCAM-1, PECAM-1, fibrinogen, fibronectin, vWF, LAP-TGF-β, thrombospondin	Monocyte, macrophage, DC, neutrophil	Migration of monocyte, macrophage and neutrophil, phagocytosis of DC and macrophage[97,98]

CR3, complement receptor-3; DC, dendritic cell; HML-1, human mucosal lymphocyte antigen-1; ICAM-1, intercellular adhesion molecule-1; LAP–TGF-β, latency-associated peptide–transforming growth factor-β complex; LFA-1, lymphocyte function-associated antigen-1; LPS, lipopolysaccharide; LPAM-1, lymphocyte Peyer's patch adhesion molecule-1; Mac-1, Macrophage-1 antigen; MAdCAM-1, mucosal addressin cell adhesion molecule-1; NK cell, natural killer cell; PECAM-1, platelet endothelial cell adhesion molecule-1; TLN, telencephalin; Treg, regulatory T cell; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; vWF, von Willebrand factor.

cytoplasmic tails of α and β subunits undergo significant separation and the extracellular parts stand up, the high-affinity conformation is generated.[6,10] In recent years, growing evidence suggests that both external and internal mechanical forces play important roles in integrin activation and bidirectional signalling. Fluid shear stress is one major external force that exerts on integrins in circulating leucocytes or those in transendothelial migration process. In contrast, when the cytoplasmic tails of integrins interact with different signalling molecules inside leucocytes, such

as talin, kindlins, vinculins and actin, tension or internal force is generated.[11] It has been reported that integrin $\alpha_5\beta_1$ is activated by tension force generated between the extracelluar fibronectin-coated surface and the intercellular cytoskeleton.[12] Other reports also shed light on our understanding of the connection between chemical signalling and the force mechanics of the integrin network.[13] The catch bond formation in the activation of the integrin headpiece is another example of an external force to activate integrins.[14]

Except for the role of external and internal mechanical forces and integrin conformational changes in affinity modulation, integrin has also been shown to form clusters or accumulate at one part of the cell to increase its avidity. In resting T lymphocytes, integrin is distributed evenly on the cell surface. After antigen activation, integrin, especially LFA-1, accumulates at the interface between a T cell and an antigen-presenting cell (APC), resulting in high avidity to enhance ligand binding.[15] Not only is LFA-1 accumulated at the interface of a T–APC conjugate, but it is also highly rearranged, together with other important T-cell surface receptors such as T-cell receptor (TCR)/CD3, to form the immunological synapse that is also termed supramolecular activation cluster (SMAC). Engaged TCRs translocate to the centre of the contact area to form the central SMAC and a ring of LFA-1 forms the peripheral SMAC with the cytoskeleton protein talin. Although the role of the immunological synapse formation in T-cell activation is still unclear, it is generally accepted that the immunological synapse facilitates the translocation of cytolytic granules during the killing of targets by cytolytic T lymphocytes or natural killer cells.[16,17] Similarly, LFA-1 also contributes to the formation of virological synapses that enhance the transmission of viruses, such as human T-cell lymphotropic virus 1 or HIV-1 between infected and non-infected cells.[18]

Molecular mechanisms of integrin activation

To bind to integrin ligands, integrin needs to be converted to an active state. Activation of integrin is a highly regulated process. Stimuli received from cell surface receptors, including TCR or B-cell receptors, and chemokine receptors, could generate intracellular signals to activate integrins; this is termed 'inside-out' signalling. On the other hand, the binding of integrin extracelluar domains to ligands or other agonists (stimulatory antibody, PMA, Mg^{2+} or Mn^{2+}), and physiological force exerted on the bond, could initiate conformational change of the integrin, which then sends biochemical and mechanical signalling into the cell to regulate multiple cellular functions; this is termed 'outside-in' signalling.[12,13] In T cells, integrin bidirectional signals lead to the formation of the immunological synapse, stabilization of T-cell–APC contact to facilitate T-cell activation, proliferation and cytokine secretion (e.g. interleukin-2, interferon-γ).[19–21] In macrophages, integrin activation induces cytoskeletal rearrangement during the process of phagocytosis, cytokine mRNA stabilization (e.g. interleukin-1β) and cell differentiation.[22] Integrin signalling also enhances neutrophil degranulation and activation of NADPH oxidase, leading to production of reactive oxygen species,[23] or induces polarization of cytolytic granules in natural killer cells or cytolytic T lymphocytes.[24] In the following discussion, we will describe those key effectors involved in integrin bidirectional signalling pathways, with particular attention to the signalling molecules in T lymphocytes.

Initiation of integrin activation by early TCR signalling

After the TCR/CD3 complex is engaged with the MHC–peptide complex, Src kinase (lymphocyte-specific protein tyrosine kinase; LCK) is phosphorylated and activated, leading to phosphorylation of immunoreceptor tyrosine-based activation motifs on the TCRξ/CD3 chains. Kinase ζ-associated protein of molecular weight 70 000 (ZAP-70) is recruited to the TCR/CD3 complex and is phosphorylated by LCK. Activated ZAP-70 then phosphorylates a number of downstream adaptors, including linker for activation of T cells (LAT) and Src homology 2 (SH2) domain-containing leucocyte protein of molecular weight 76 000 (SLP-76) (Fig. 1). Elevated levels of LCK in cloned cytolytic T cells markedly increase cytolytic activity and enhance LFA-1 expression levels with increased cell binding to the ligand intercellular adhesion molecule 1 (ICAM-1).[25] In LCK-deficient Jurkat cells (i.e. JCaM1.6 cells) or in Src kinase inhibitor PP2-treated Jurkat cells, CD3 ligation-induced adhesion to ICAM-1 is dramatically reduced.[26] These studies suggest that LCK is a positive regulator for integrin activation. Similarly, ZAP-70-deficient Jurkat cells fail in TCR-induced integrin β_1-mediated adhesion and the kinase activity of ZAP-70 required for LAT phosphorylation is crucial for integrin activation.[27] This fits with the defective integrin activation and adhesion in LAT-deficient Jurkat cells. Further, LAT is associated directly or indirectly with a number of key signalling proteins, including phosphatidylinositol 3-kinase, the inducible T-cell kinase (ITK), SLP-76, and phospholipase C-γ1 (Fig. 1). These kinases, adaptors or enzymes have been implicated to play critical roles in TCR-induced 'inside-out' signalling for integrin activation.[28]

The SLP-76–VAV1 complex

The protein SLP-76 is an important adaptor protein downstream of the LAT–Gads (Grb2-related adaptor downstream of Shc) complex in T cells (Fig. 1) and has been demonstrated as a positive regulator for T-cell development and cell activation. SLP-76-deficient mice show a

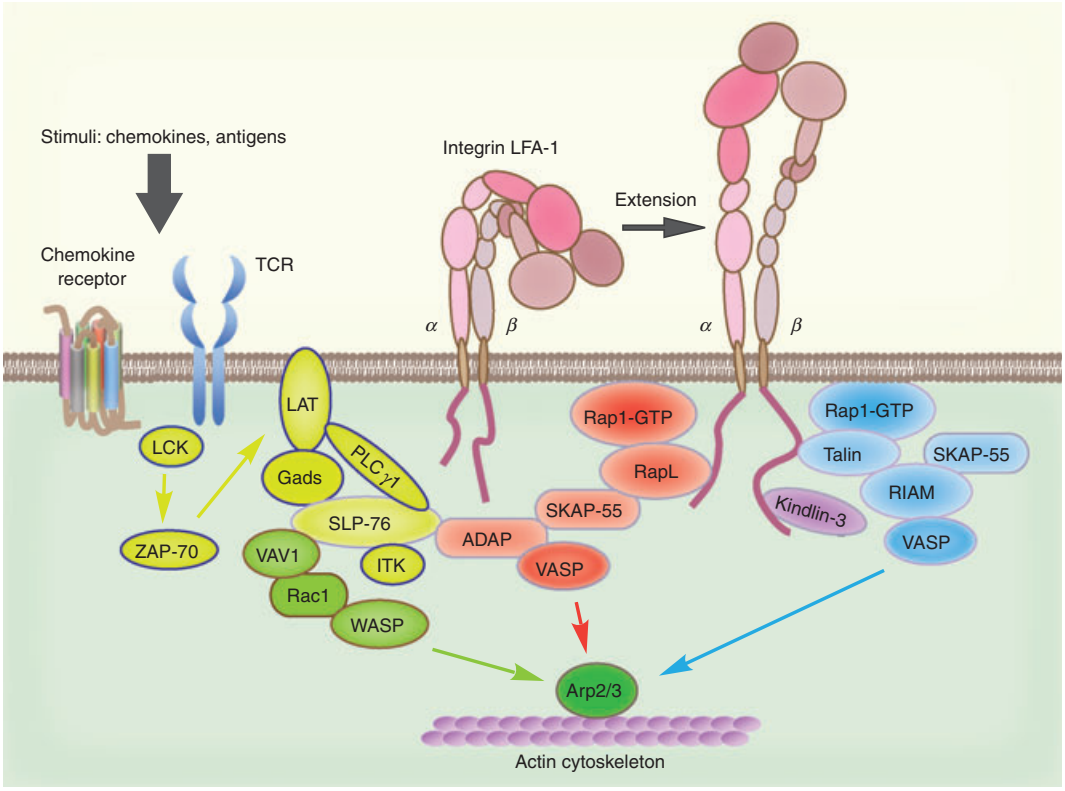

Figure 1. Key effectors in integrin activation and signalling pathways in T lymphocytes. After T-cell receptor (TCR)/CD3 or chemokine receptor is activated, Src kinases such as lymphocyte-specific protein tyrosine kinase (LCK) are phosphorylated and activated, leading to phosphorylation of TCR/CD3. Kinase ζ-associated protein of molecular weight 70 000 (ZAP-70) is then recruited to the TCR/CD3 complex and is phosphorylated by LCK. Activated ZAP-70 phosphorylates a number of downstream adaptors, including linker for activation of T cells (LAT) and SH2-domain-containing leucocyte protein of molecular weight 76 000 (SLP-76) (shown in yellow). Being a central scaffolding protein, SLP-76 is associated with a guanine-nucleotide exchange factor (GEF) Vav1, while VAV1 activates the GTPase Rac1, which interacts with WASP (Whiskott-Aldrich syndrome protein) and activates the ARP2/3 (actin-related protein-2/3) complex (shown in green). SLP-76 also interacts with ADAP (adhesion and degranulation promoting adapter protein), while ADAP directly binds Src kinase-associated protein of molecular weight 55 000 (SKAP-55) and VASP (WASP-family verprolin-homologous protein). SKAP-55 binds and brings RapL to membrane located GTP-Rap1, resulting in the direct interaction of RapL to lymphocyte function-associated antigen 1 (LFA-1) to increase cell adhesion (shown in red). SKAP-55 also constitutively interacts with Rap1–GTP-interacting adapter molecule (RIAM). The ability of RIAM binding to VASP and talin suggests that RIAM promotes integrin activation through effects on the actin cytoskeleton, particularly the interaction of talin with integrin cytoplasmic tails (shown in blue). Other actin-associated proteins kindlin and paxillin have also been identified to regulate integrin activation.

T-cell developmental block at the double-negative stage, whereas the SLP-76-deficient T-cell line shows impaired phosphorylation of phospholipase C-γ1 and defective Ras pathway activation.[29–31] Importantly, SLP-76 has been implicated in the regulation of integrin adhesion in both 'inside-out' signalling and 'outside-in' signalling in multiple cell types. SLP-76-deficient T cells could not adhere to integrin β_1 ligand fibronectin after TCR stimulation via the 'inside-out' signalling. Further, in response to ligand-induced 'outside-in' signalling, SLP-76-deficient platelets fail to spread on integrin β_3 ligand fibrinogen-coated plates,[32,33] and SLP-76-deficient neutrophils fail to spread and produce reactive oxygen intermediates after integrin ligand simulation.[34] Interestingly, the upstream effectors LAT and Gads do not seem to play a role because the Gads-binding domain of SLP-76 seems to be dispensable for platelet spreading on fibrinogen, and LAT-deficient platelets aggregate and spread normally in response to integrin stimulation in the 'outside-in' signalling.[35]

As a central scaffolding protein, SLP-76 is associated with a guanine-nucleotide exchange factor (GEF) Vav1 after being phosphorylated by ZAP-70 and SYK.[36–38] Similar to the role of SLP-76, Vav1 mediates integrin β_1 and β_2 activation in T cells, neutrophils and platelets via both 'inside-out' and 'outside-in' pathways. Vav1-deficient cells are impaired in cell adhesion, spreading and production of reactive oxygen intermediates in response to integrin ligand stimulation in the 'outside-in' signalling.[39–42] Also, Vav1 mediates TCR-induced integrin clustering and T–APC conjugate formation via 'inside-out' signalling.[41] As a GEF, Vav1 activates the GTPase Rac1, which regulates adhesion by directly controlling the balance between

actin-mediated protrusion and myosin II-mediated contraction through interacting with the WASP/WAVE complex and activating the ARP2/3 complex (Fig. 1).[43–45] Other GEFs including DOCK180 (dedicator of cytokinesis 180), DOCK8 also regulate integrin adhesion, which activate the GTPase Rac1 or Cdc42.[46]

The SLP-76–ADAP–SKAP-55 complex

Upon activation, SLP-76 also interacts with adhesion and degranulation promoting adaptor protein (ADAP) via its phosphorylated tyrosines.[47] The SLP-76–ADAP interaction regulates integrin-initiated 'outside-in' signalling.[48] Disruption in the interaction between SLP-76 and ADAP blocks T-cell spreading and migration in the ligand ICAM-1-coated surface.[49,50] Similar to 'outside-in' signalling in other cells, the upstream LAT–Gads complex is not required for the SLP-76–ADAP module-induced 'outside-in' signalling in T cells.[49] In TCR-induced 'inside-out' signalling, ADAP-deficient T cells have shown impaired integrin β_1 and β_2-mediated cell adhesion, LFA-1 clustering and the formation of T–APC conjugates. This defect in adhesion is accompanied by reduced T-cell proliferation and interleukin-2 production.[51–53] Defects in T-cell selection have also been documented in certain ADAP-deficient transgenic models expressing a single TCR.[54] ADAP binds directly to Src kinase-associated protein of molecular weight 55 000 (SKAP) by the interaction of the SKAP-55 SH3 domain to a proline-rich region in ADAP or the interaction of the ADAP SH3c domain to a tyrosine-based RKXXYXXY motif in SKAP-55 (Fig. 1).[55–58] SKAP-55 is expressed in a restricted manner in T cells as a positive regulator for integrin activation, T-cell adhesion and T–APC conjugate formation.[51,59,60] The role of SKAP-55 in the regulation of integrin activation could not be replaced by its homologue protein SKAP-55-related (SKAP-55R, also termed SKAP-55 Hom).[59,61] Disruption of the ADAP–SKAP-55 module by deletion of the SKAP-55 SH3 domain or the ADAP proline-rich domain impairs formation of T–APC conjugates, LFA-1 adhesion and may prevent the membrane translocation of small G protein Rap1, a key player of integrin activation.[51,62]

The SKAP-55–RapL/RIAM–Rap1 complex to integrins

Although important for integrin activation, SLP-76, ADAP and SKAP-55 do not interact with integrin directly. Recently, we have identified that the ADAP–SKAP-55 module comprises a complex with the Rap1–RapL module after TCR stimulation. It has been demonstrated that RapL binds activated Rap1 after TCR or chemokine stimulation, and this interaction brings RapL close to the cell membrane to allow direct binding of the RapL to the cytoplasmic domain of the α_L chain of LFA-1 (Fig. 1). RapL-deficient T or B cells are defective in cell adhesion and trafficking. We found that the N-terminal domain of SKAP-55 binds to the C-terminal SARAH domain of RapL, resulting in the formation of an SKAP-55–RapL–Rap1 complex that binds to LFA-1 and increases adhesion to ICAM-1. The Rap1–RapL complex formation and LFA-1 binding fail to occur in SKAP-55-deficient T cells. By contrast, chemokines SDF1 and CCL21 induce normal migration of SKAP-55-deficient T cells.[63] Hence, SKAP-55 appears to serve as a specific adaptor to couple the TCR with the activation of the Rap1–RapL module for integrin adhesion.

Another Rap1–GTP binding partner is Rap1–GTP-interacting adapter molecule (RIAM). Over-expression of RIAM increases cell spreading, lamellipod formation, integrin activation and adhesion.[64] It has been shown that RIAM constitutively interacts with SKAP-55, and that the ADAP–SKAP-55 module promotes the membrane location of the RIAM–Rap1 module following TCR activation to facilitate integrin activation.[65] In addition, the ability of RIAM to bind to profilin, Ena/VASP proteins and talin suggests that RIAM promotes integrin activation through effects on the actin cytoskeleton, particularly the interaction of talin with integrin cytoplasmic tails (Fig. 1).

Talin and kindlin interactions to integrins

Talin is a cytoskeletal protein consisting of an N-terminal FERM domain (protein4.1, ezrin, radixin and moesin) with three subdomains (F1, F2, F3), which binds integrin cytoplasmic tails (Fig. 1) and a large C-terminal rod domain that binds actin.[66,67] The F3 subdomain contains a phosphotyrosine-binding (PTB) domain that binds the integrin β subunit tail at the membrane-proximal NXXY site.[67] Talin is enriched at the leading edge of chemokine-stimulated lymphocytes and in the immunological synapse together with LFA-1, vinculin and F-actin.[68] Hence, talin acts as a bridge to link the extracellular matrix and the actin skeletal network.

Kindlin is another essential player that binds differently to the integrin β subunit tail at the membrane-distal NXXY site and activates integrin (Fig. 1). Kindlin is named after the Kindler syndrome which is a kind of skin blistering disease caused by a *kindlin-1* gene mutation.[69] The kindlin family has three members, including kindlin-1 (Unc-112-related protein 1, URP1), kindlin-2 (Mig2) and kindlin-3 (URP-2), which all have a conserved FERM domain composed of four subdomains. Among them, kindlin-3 is expressed exclusively in cells of haematopoietic origin. The FERM subdomain 2 in kindlin-3 is featured by a pleckstrin homology domain that is involved in membrane binding,[70] and subdomain 3 in kindlin-3, which binds the distal motif of integrin β_1, β_2 and β_3 tails.[71–73] Mutations in kindlin-3 result in defective integrin activation in leucocytes and platelets and lead to leucocyte adhesion deficiency III.[74] Kindlins are not suffi-

cient to induce integrins to a high-affinity state, but they can promote the binding of talins to integrin tails. Talin is also not sufficient to increase integrin affinity without the aid of kindlin.

Other actin-associated proteins have also been identified to interact with integrins. Paxillin is a cytoskeletal phosphotyrosine-containing protein and binds directly to the cytoplamic domain of integrin α_4.[75] The interaction is regulated in a protein kinase A-dependent manner. Phosphorylation of the α_4 cytoplasmic domain at serine988 leads to release of paxillin from integrin.[76] It mediates initial capture and rolling interactions during leucocyte migration on vascular cell adhesion molecule 1-expressing and mucosal addressin cell adhesion molecule-1-expressing vascular endothelium.[77]

Conclusions

Integrins play many essential roles in leucocytes and many key players in both 'inside-out' and 'outside-in' pathways have been well characterized since the middle 1980s. However, challenging questions remain. One major question is how different integrins coordinate with other surface receptors in different cell types to regulate cellular functions when responding to various agonists including antigens, chemokines, selectins and others. For example, the MHC–peptide complex binding to TCR induces 'inside-out' signalling for LFA-1 activation and LFA-1 co-ligation further acts as a 'co-stimulator' for T-cell proliferation, calcium mobilization and lytic effect cell induction.[78] After binding of the bacterial product lipopolysaccharide to Toll-like receptor 4, integrin Mac-1 (CD11b/CD18) could also be activated in macrophages. However, in contrast to the positive role of LFA-1 in T-cell activation, integrin Mac-1 plays a negative role to reduce Toll-like receptor-mediated signalling and limits inflammation.[79] Further, new functions of integrins in leucocytes are emerging. Integrin $\alpha_4\beta_7$ in mucosal T cells binds directly with the V2 loop of gp120 in HIV-1, which results in rapid activation of LFA-1 to facilitate the formation of virological synapses and efficient cell-to-cell spreading of HIV-1. Blocking the interaction of integrin $\alpha_4\beta_7$ with gp120 via a peptide could significantly reduce HIV-1 entry into T cells.[80] ITK, which regulates integrin activation, can enhance HIV-1 entry and transmission between cells.[81] Integrin $\alpha_E\beta_7$ (CD103) has also been identified in regulatory T (Treg) cells but plays no mandatory role for Treg-cell-mediated control of colitis.[82] Signalling proteins Rap1 and protein kinase C-θ (PKC-θ) which affect integrin activation might regulate Treg-cell function.[83,84] With more detailed understanding of the role of different integrins in different cell types, we would target specific integrins with blocking antibodies, RGD (arginine-glycine-aspartic acid) peptides or small molecules in the treatment of various diseases. For example, blocking antibody to α4-integrin has shown some degree of success in multiple sclerosis and in inflammatory bowel disease.[9] However, there are some remaining concerns, including the possibility that blocking integrin function would generally compromise the immune system's ability to fight against infection or that diseases might relapse upon cessation of blockade of integrins. It is therefore important to understand the underlying molecular mechanism of how integrin function is regulated, and this might provide us with new specific targets through which to treat integrin-related diseases.

Acknowledgements

This work was supported by grants from the Ministry of Science and Technology of China (2011CB505005 and 2012CB910800), National Natural Science Foundation of China (31070778), the Chinese Academy of Sciences and Shanghai Science and Technology Committee (11PJ1410700).

Disclosures

The authors have no conflicts of interest to disclose.

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