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SKAP-55, SKAP-55-related and ADAP adaptors modulate integrin-mediated immune-cell adhesion

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Abstract

Integrin adhesion is essential for aspects of immune function, including antigen presentation and migration in lymph nodes, germinal centers and sites of inflammation. Antigen receptors on B and T cells generate ‘inside-out’ signals for increased integrin clustering and adhesion. Although upstream components of B-cell-receptor or T-cell-receptor signaling are needed, the identity of key downstream effectors that mediate integrin adhesion is only just emerging. New candidates include immune-cell-specific adaptor proteins ADAP, SKAP-55 and SKAP-55-related (SKAP-55R). SKAP-55 has recently been identified as an effector in T cells in SKAP-55-deficient mice, whereas SKAP-55R is needed for B-cell adhesion. ADAP is required for SKAP-55 and SKAP-55R protein stability. SKAP-55 and SKAP-55R have unexpectedly specialized roles in T- and B-cell adhesion of the immune system.

TCR signaling and LFA-1 activation

Integrins on the surface of immune cells mediate multiple functions in the immune system. Immune cells are among the fastest moving cells, achieving speeds of $15 \mu\text{m sec}^{-1}$. Adhesion is needed for migration of T and B cells to different tissues and sites of inflammation, for movement in lymph nodes and germinal centers and during the conjugation of T cells with antigen-presenting cells (APCs) [1,2]. Of the 12 integrins that are expressed on lymphocytes, $\beta 2$ integrins are preferentially expressed. Of these, $\alpha\text{L}\beta 2$ [leukocyte function-associated antigen-1 (LFA-1), also termed CD11a (αL chain of LFA-1)–CD18 ($\beta 2$ chain of LFA-1)] binds to the ligand ICAM-1,2 and 3 (intracellular adhesion molecules 1,2 and 3). ICAMs are expressed on endothelial cells that line blood vessels and on the surface of APCs. T-cell migration along the inner walls of blood vessels is initially slowed by a weak interaction between selectins (L-selectin, P-selectin and E-selectin) and ligands such as glycosylation-dependent cell-adhesion molecule 1 (GlyCAM-1), CD34 and mucosal addressin cell-adhesion molecule-1 (MadCAM-1) on endothelial cells. These interactions create a slowing in the form of ‘rolling and tethering’ along endothelial cells. This, in turn, enables firmer binding between LFA-1 and ICAM1, which is needed for transmigration through high endothelial venules (HEVs). This step is mediated through junction-adhesion molecules, including JAM1 (junctional-adhesion molecule 1), CD31 [also known as PECAM-1 (platelet-endothelial-cell-adhesion molecule-1)] and CD44 (homing function and Indian-blood-group system) (Figure 1a). The process of transmigration enables cells to move to sites of inflammation and specific tissues for an encounter with APCs. APCs process foreign peptides to T cells, which are presented

in the context of a major histocompatibility complex (MHC) (peptide-MHC) (Figure 1b). Peptide-MHC, in turn, binds to the antigen-receptor [T-cell receptor (TCR)-CD3 complex] on T cells, which activates cells and their ability to elicit immune functions, including cytokine production and T-cell cytotoxicity.

For integrins to bind to ligands such as ICAM1, they need to be converted to an active state. This activation event involves two steps: one involving a conformational change that increases affinity and another that involves receptor clustering, which increases avidity. LFA-1 is in a low-adhesive state on resting cells. Activation can be induced by chemokine receptors (including Cys-Xaa-Cys motif receptor 4, Cys-Cys motif receptor 5 and others), the TCR-CD3 complex and by co-receptors such as cytotoxic T-lymphocyte-antigen 4. Affinity changes occur by the unfolding of the receptor (i.e. the 'jack-knife' model), which produces 'intermediate-high' affinity versions of the integrin [3-5]. Clustering of these unfolded receptors then leads to a major increase in avidity. Although the inter-relationship between affinity and avidity changes is not entirely understood, intermediate-avidity versions of integrins are found at the leading edge of the migrating T cell, whereas high-affinity LFA-1 seems to be restricted to the mid-cell focal zone and controls ICAM-1 attachment [6]. These higher-affinity forms are presumably needed for a firm grip on substrates.

The initial contact between a T cell and an APC is made by random encounters and by chemokines that can partially activate integrins on T cells for contact (Figure 1b). This initial contact enables ligation of the TCR complex that then induces 'inside-out' signals, thereby activating high-avidity adhesion. When a stable conjugate is formed, an interface between T cells and APCs occurs, termed the 'immunological synapse' (IS). Chemokine receptors are recruited to and sequestered at the IS, so that T cells might become insensitive to other chemotactic gradients [7]. Chemokines can enhance this process and increase the longevity of the adhesion between integrins and ligand and the longevity of the conjugation. This firmer contact enables TCR interaction with the MHC-peptide complexes via serial ligation. The process also involves the formation of signaling clusters (termed microclusters) that eventually coalesce to form the supramolecular activation cluster (SMAC) (Figure 1b). The SMAC is ~5-10 μm across. Engaged TCRs in microclusters become translocated to the center of the contact area to form the central SMAC (cSMAC) and a ring of LFA-1 forms the peripheral SMAC (pSMAC) (Figure 1b). LFA-1 binds and recruits the cytoskeleton protein talin to the pSMAC structure. Although microclusters have been clearly implicated in signaling with increased tyrosine phosphorylation, the role for the SMAC has been unclear. LFA-1 mediates adhesion before the formation of this structure, and most tyrosine substrate phosphorylation occurs before and outside the SMAC. However, the SMAC has roles in effector functions, such as the transfer of viruses [e.g. human T-cell lymphotropic virus 1 (HTLV-1)] between infected and non-infected cells [8] and the translocation of cytolytic granules during the killing of targets by cytolytic T lymphocytes (CTLs), which occurs within pSMAC [9].

Given the importance of integrin-mediated adhesion to numerous aspects of immunity, there has been major interest in defining the nature of the intracellular signaling pathway(s) responsible for TCR-mediated 'inside-out' signaling. One of the earliest events of TCR ligation is the activation of protein tyrosine kinases (PTKs) p56lck and p59fyn [10,11]. The CD4 and CD8 co-receptors bind to p56lck and are brought into the proximity of the TCR complex owing to their binding to non-polymorphic regions of the MHC antigens [11]. This, in turn, leads to the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) within the CD3 and T-cell receptor ζ chains and recruit the ZAP-70 (ζ -chain-associated protein kinase-70) kinase [12]. Subsequent phosphorylation of ZAP-70 by p56lck within the activation loop of the kinase domain activates ZAP-70, which, in turn,

phosphorylates so-called ‘adaptor’ proteins or molecular scaffolds. These proteins lack enzymatic activity and, instead, carry binding modules or sites for the assembly of supra-molecular complexes [13-15]. Of these, LAT (linker for activation of T cells), GADS (Grb2-related adaptor down-stream of Shc) and SLP-76 (Src homology 2 domain-containing leukocyte protein of 76 kDa) are key mediators. ZAP-70 phosphorylates tyrosines on LAT, which recruits GADS that, in turn, bind to SLP-76. This complex also recruits the kinase ITK (interleukin-2-inducible T-cell kinase), which is needed for the phosphorylation of the phospholipase C γ 1 and the mobilization of intracellular calcium [15,16]. Disruption of upstream components such as p56lck, ZAP-70, LAT, SLP-76 or GADS-SLP-76 interaction results in a generic impairment of LFA-1-mediated adhesion [17-19] (Figure 2). A recent study has shown that a polypeptide identical to the site on SLP-76 that binds to GADS can also inhibit TCR-induced integrin adhesion and thymocyte development [20].

Despite the importance of these general upstream events, the quest over the past few years has been to identify the key downstream effector adaptors of the ‘inside-out’ adhesion pathway. A major advance in this area has recently come with the discovery of immune-cell adaptors ADAP [previously known as Fyn T-binding protein (FYB) or SLP-76-associated protein (SLAP)] and SKAP-55 (src kinase associated protein of 55 kDa). In this review, we discuss the current understanding of ADAP, SKAP-55 and SKAP-55-related [(SKAP-55R), also known as SKAP-55 homolog (SKAP-HOM)] in the regulation of integrin activation and the related biological function in immune cells.

ADAP

ADAP has a proline-rich domain, multiple tyrosine sites, two putative nuclear-localization sites (NLS), two SH3 domains and an Ena (enabled) and vasodilator-stimulated phospho protein (VASP) homology 1 (EVH1) domain-binding site (Figure 3). ADAP is expressed primarily in many hematopoietic cells, but not in B cells (Table 1). Two isoforms of ADAP at 120 and 130 kDa (ADAP-120 and ADAP-130, respectively) exist. It has been shown that the 130-kDa isoform contains an extra insertion of 46 amino acids and is preferentially expressed in peripheral T cells [21]. After TCR-CD3 stimulation, two Tyr-Asp-Asp-Val (YDDV) motifs at residues 595-598 and 651-654 in ADAP are phosphorylated and function as binding sites for the SH2 domain of SLP-76 [22]. Analogous motifs have subsequently been documented in the hematopoietic progenitor kinase 1 (HPK1) [23-25]. Mutation of either the Tyr595 or Tyr651 site in ADAP impairs T-cell activation [26]. An alternative Tyr559-Gly-Tyr-Ile site has also been claimed, but its relevance has yet to be confirmed [22,27,28]. By contrast, a Tyr-Asp-Gly-Ile (YDGI) motif at residues 625-628 binds the SH2 domain of src kinase p59^{fyn} [21]. Fyn preferentially phosphorylates the YDDV sites and Fyn-deficient T cells show a major reduction in ADAP phosphorylation [21,22,28,29]. ADAP also binds constitutively to SKAP-55 or SKAP-55R [30-33]. Binding to ADAP primarily occurs via SKAP-55 SH3 domain recognition of a proline-rich region in ADAP (not phospho-tyrosine, such as in the case of SH2 domains). The secondary interaction is mediated via the binding of the ADAP SH3-like domain [also termed helically extended SH3 domain (hSH3 domain)] to a tyrosine-based Arg-Lys-Xaa-Xaa-Tyr-Xaa-Xaa-Tyr (RKxxYxxY) motif in SKAP-55, which is modulated by TCR stimulation [30-33]. The hSH3 domain in ADAP has also been reported to bind to phospholipids that are essential for ADAP-mediated migration and adhesion of T cells [34,35]. The degree to which the ADAP hSH3 domain binds the RKxxYxxY motif and/or phospholipids is the subject of debate [31,34,35]. Although SKAP-55 SH3 domain interaction with ADAP predominates, mutation of the RKxxYxxY motif in SKAP-55 can also disrupt adaptor function [36].

In addition to its binding to SLP-76, SKAP-55 and SKAP-55R, ADAP is connected to the cytoskeleton as a result of the binding of ADAP to the EVH1 domain in Ena and VASP

(enabled and vasodilator-stimulated phospho protein) via its EVH1 binding site [37,38]. VASP, in turn, binds profilin, a small actin-monomer-binding protein. In support of its importance, ActA peptides derived from *Listeria monocytogenes* that bind the Ena and VASP family can interfere with the remodeling of the actin cytoskeleton upon TCR ligation [37]. This approach has been limited by the fact that the ActA peptides used also interfere with other EVH1 domain interactions. By contrast, the mutation of the Phe/Leu/Trp/Tyr-Pro-Pro-Pro-Pro motifs in ADAP had no obvious effect on adhesion or T-cell-APC conjugation [39]. This is in contrast to the mutation of the YDDV sites (termed M12) for the binding of SLP-76 that disrupts TCR-driven adhesion, peripheral SMAC formation and IL-2 production [39]. In another connection, ADAP has been reported to co-precipitate with microtubule-motor-protein dynein from activated Jurkat cells, and localizes at the IS and SMAC [39,40] (Table 1).

Although the function of ADAP as a positive or negative regulator of cell activation was initially unclear [21,22,28], data from ADAP-deficient T cells have confirmed its positive function [41,42]. Although these ADAP-deficient mice have a normal presence of T-cell subsets (such as CD4 and CD8), ADAP-deficient T cells have shown impaired $\beta 1$ and $\beta 2$ integrin-mediated cell adhesion and LFA-1 clustering after anti-CD3 stimulation. This defect in adhesion is accompanied by reduced proliferation and IL-2 production [41,42]. Whereas gross changes in thymic selection have not been observed, defects in selection have been documented in certain transgenic models expressing a single TCR [43]. Overall, the observations of ADAP-deficient mouse have pointed to a key role for this adaptor in facilitating 'inside-out signaling'.

SKAP-55

The two studies on the *ADAP*^{-/-} mouse were quickly followed by the key and unexpected finding that ADAP-deficient Jurkat cells also lack SKAP-55 expression [44]. This finding has also been confirmed in primary T cells [45,46]. ADAP has been found to regulate the stability of SKAP-55 by influencing the rate of SKAP-55 proteolysis (Figure 2). It binds with high stoichiometry to ADAP such that the depletion of ADAP from the cell lysates depletes most of SKAP-55 and vice versa (i.e. ~70% of endogenous ADAP binds SKAP-55) [29,32]. This finding has raised the important question of whether the phenotype of ADAP-deficient T cells is caused by the lack of ADAP, SKAP-55 or both.

SKAP-55 or Skap1 (NCBI assignment; <http://www.ncbi.nlm.nih.gov>) contains a pleckstrin homology (PH) domain, a C-terminal SH3 domain and several tyrosine-based residues and is expressed exclusively in T-lymphocytes, mast cells and macrophages, but not in B cells [30,47] (Table 1). Initial evidence implicating SKAP-55 in the regulation of T-cell adhesion came with retroviral transduction of T cells. Over-expression of SKAP-55 in mouse primary T cells increases integrin-mediated adhesion and conjugates formation between T cells and APCs [48]. SKAP-55 also co-localizes with F-actin at the T cell-APC synapse. Deletion of the SKAP-55 SH3 domain, the ADAP SH3c domain or the ADAP proline-rich domain downregulates conjugation formation and blocks LFA-1 clustering formation [46,48]. Lastly, the use of small interfering RNAs (siRNA) to knock-down SKAP-55 expression in T-cell lines interferes with adhesion and LFA-1 clustering without affecting related SKAP-55R or ADAP expression [46,49]. This indicates that SKAP-55R cannot compensate for the loss of SKAP-55 in the regulation of T-cell function.

SKAP-55-deficient mice

Despite the results discussed here, there is little information available on the function of SKAP-55 in primary T cells and systemic mouse immunity. A breakthrough has come with the generation of the SKAP-55-deficient mouse, which shows normal levels of ADAP

expression [45]. This has enabled the study of SKAP-55 function independently of ADAP and has shown that SKAP-55 has no obvious role in regulating ADAP expression. The chaperone function of ADAP in regulating SKAP-55 degradation is, therefore, uni-directional.

The phenotype of the SKAP-55-deficient mouse has determined several things. First, SKAP-55-deficient T cells show a comparable degree of impaired integrin clustering and adhesion, interleukin-2 and interferon- γ cytokine production and proliferation, as in ADAP-deficient T cells [45]. T cells lacking SKAP-55 exhibit more transient conjugation times than wild-type cells in response to super-antigen staphylococcal enterotoxin A (SEA)-bearing dendritic cells and a reduced localization of TCR-CD3 microclusters at the IS. As with the *ADAP*^{-/-} mouse, SKAP-55-deficient mice have also shown little change in thymic development, or in the appearance of peripheral T, B and natural killer (NK) cells. The similarity of the ADAP-deficient (i.e. also a lack of SKAP-55) and SKAP-55-deficient mice strongly implicated SKAP-55 as the effector within the ADAP-SKAP-55 module in the regulation of integrin activation and cell adhesion (Figure 2).

The second surprise from studies on *SKAP-55*^{-/-} and *ADAP*^{-/-} T cells was that the requirement for the adaptors is not absolute and varies with the strength of the TCR signal [45]. Previous studies have reported a clear dependency on ADAP; however, this was observed using a single low concentration of anti-CD3 antibody [41,42]. With increasing concentrations of anti-CD3, fewer cells have been found to be dependent on SKAP-55 or ADAP. Cell-cycle analysis has shown that once a few of ADAP-deficient or SKAP-55-deficient cells enter the first cycle of cell division, they progress to the second and third cycle in a manner comparable to wild-type cells [45]. When anti-CD3 is used at 10 μgml^{-1} , adhesion occurs normally without the need for SKAP-55 or ADAP. Consistent with this study, ADAP-deficient T cells have also shown defective cell activation when treated with limiting antigen doses *in vivo*, whereas ADAP has been shown to be dispensable when stimulated with high doses of antigen [50]. Together, these observations have made the important point that an alternative pathway exists by which the TCR can upregulate LFA-1 adhesion independently of ADAP or SKAP-55. The identity of this second pathway remains unknown. The result also predicts that ADAP and SKAP-55 might have a greater role in response to lower affinity peptides (i.e. weaker TCR signal), whereas the alternative pathway might mediate signaling in response to stronger TCR signals (i.e. higher-affinity peptide).

SKAP-55R-deficient mice

The observation that the defect in the SKAP-55-deficient mice is restricted to T cells has complemented the observation that the loss of the related homolog SKAP-55R shows a defect primarily in B cells, and not in T cells [51] (Table 1). This fits with the notion that antigen-receptors on T and B cells employ related, but distinct, adaptors to activate integrin adhesion. SKAP-55R has the same overall structure as SKAP-55, with a PH domain, multiple tyrosine phosphorylation sites and a SH3 domain [30,33] (Figure 3). SKAP-55R and SKAP-55 show 44% identity with the greatest conservation in the PH and the SH3 domains. The tyrosine motif Tyr-Glu-Val-Leu in SKAP-55 exists as Tyr-Glu-Glu-Leu in SKAP-55R, and both motifs could potentially serve as src kinase SH2-domain-binding sites. The major structural difference between SKAP-55 and SKAP-55R is in the N-terminal region in which SKAP-55R has a well-defined coiled-coil domain (residues 20-75). Coiled-coils are formed by the coiling of two right-handed helices around each other with a slight left-handed super-helical twist [52]. SKAP-55R-deficient B cells have shown strongly reduced adhesion to ICAM-1 and fibronectin, whereas other aspects of B-cell-receptor (BCR)-mediated signaling seem normal. T and B lymphocyte differentiation is normal in

SKAP-55R-deficient mice. *In vivo*, the loss of SKAP-55R has resulted in a less severe clinical course of experimental autoimmune encephalomyelitis, whereby immunization occurs with the encephalitogenic peptide of MOG (myelin oligodendrocyte glycoprotein). This is accompanied by reduced serum MOG-specific antibodies and lower MOG-specific T-cell responses [51]. These effects are consistent with a role for integrins in elucidating adhesion and migration for response. Whether the dependence on SKAP-55R varies with the strength of the antigenic signal, as observed in SKAP-55 mice, has yet to be determined. Overall, these data indicate that the related adaptors SKAP-55 and SKAP-55R have specialized roles in T and B cells of the immune system.

ADAP, SKAP-55 and SKAP-55R regulation of adhesion in other cells

In addition to T and B cells, ADAP deficiency in platelets has caused a reduction in $\alpha\text{IIb}\beta_3$ integrin activation and platelet adhesion to fibrinogen [54]. Consistent with this, ADAP-deficient mice have shown decreased clotting responses and increased bleeding. By contrast, no abnormalities have been observed in SKAP-55R-deficient platelets, whereas SKAP-55 is not expressed in platelets [53,54]. The SLP-76–ADAP–VASP–profilin complex has been identified in macrophages, and has been linked to the actin cytoskeleton re-modeling and the ability to clear bacteria [38]. Although most other bacteria are effectively cleared by the resident phagocytes, the bacteria *Yersinia* bind to receptors on the surface of macrophages and block their phagocytic function. In this case, *Yersinia* protein tyrosine phosphatase YopH is translocated into macrophages and selectively dephosphorylates ADAP and SKAP-55R, leading to reduced adhesion and blockage of phagocytosis and cytotoxic effects [55–58] (Table 1).

ADAP also localizes within the leading edge of lamelli-podia and in pseudopodia in osteoclast precursors [59]. Tyrosine phosphorylation of ADAP is induced in an integrin-dependent manner, whereby the Tyr807 in ADAP bound to the SH2 domain of c-Src and the phosphorylation of Cas by c-Src is decreased in ADAP knock-down cells [59]. Although ADAP is also expressed in NK cells, it is dispensable for NK-cell development, cytotoxicity, cytokine production and conjugate formation [60]. The full involvement of SKAP-55 and SKAP-55R in these systems has yet to be determined.

Model of the ADAP–SKAP-55 pathway in integrin adhesion

Great progress has been made in the past few years in defining the molecular basis of antigen-receptor-induced ‘inside-out’ signaling in immune cells, with increasing evidence of an effector role for SKAP-55 and SKAP-55R. In T cells, the general model involves TCR-induced phosphorylation of LAT, leading to the recruitment of GADS-SLP-76, which, in turn, recruits ADAP and SKAP-55 (Figure 2). At a minimum, ADAP operates to regulate SKAP-55 and SKAP-55R protein expression and functions as a bridge between TCR-SLP-76 and SKAP-55 effector functions. It might also modulate the actin cytoskeleton via binding to VASP and dynein. One possibility is that ADAP might increase the size of the interface of the IS between T cells and APCs. The phosphorylation of both ADAP and SKAP-55 is preferentially regulated by the src kinase p59fyn. In B cells, after phosphorylation of ITAMs in BCR, the spleen tyrosine kinase (SYK) kinase phosphorylates the SLP-76 homolog SLP-65, which then forms a complex with BTK (Bruton’s tyrosine kinase), leading to the phosphorylation of phospholipase- γ_2 (PLC γ_2) and the intracellular mobilization of Ca^{2+} [61]. In an unknown manner, this complex engages SKAP-55R, which further connects to the downstream effectors Rap1 or Rap2 to regulate cell adhesion and integrin activation in B cells [62] (Figure 2).

The nature of the downstream target of SKAP-55 remains to be determined. The GTPase Rap1 and its binding proteins RapL (regulator of cell adhesion and polarization enriched in

lymphoid tissues) and Riam (Rap1–GTP-interacting adaptor molecule) are possible candidates for this down-stream target of SKAP-55 [63–65]. One model has proposed that SKAP-55 is needed for the translocation of Rap1 to the cell surface [46,66]. However, this finding needs to be tempered by the fact that Jurkat cells used in this study have abnormally high constitutive levels of D3-lipids [67]. The SKAP-55 PH domain (which binds D3 lipids) would be expected to bind constitutively to the membranes of these cells. Furthermore, as in the case of p21ras [68], it is not clear whether Rap1 function occurs solely in the plasma membrane [69]. In fact, several reports have claimed that Rap1 function exhibits from intracellular compartments [69]. Nevertheless, it remains possible that SKAP-55 could contribute to Rap1 function via this mechanism.

Another model proposes that SKAP-55 binds directly to Riam and that this binding is needed for the ability of Riam to function in adhesion [66]. The Riam–Rap1 module has been reported to activate integrins by forming an integrin-associated complex containing talin [70]. Although intriguing, the difficulty with this model is that the binding has been proposed to occur via the Riam PH and RA (Ras association) domains. The RA domain is needed to interact with GTP-bound Rap1; an interaction that is needed for Riam function [64]. It is necessary to clarify whether the RA can bind to both SKAP-55 and Rap1 at the same time, or whether it binds separately to both in the formation of a multimeric complex. Similarly, binding to the PH domain would be expected to interfere in the ability of Riam to bind to the plasma membrane. PH domains bind to D-3 lipids and are needed for membrane localization [71]. Other future models are likely to include RapL and its modulation of adhesion. It has been demonstrated that RapL binds activated Rap1 after TCR and chemokine stimulation and RapL-deficient T or B cells are defective in cell adhesion and trafficking [63,65]. Our recent data have shown that SKAP-55 binds RapL and that this interaction is crucial for cell adhesion (M. Raab *et al.*, unpublished) (Figure 2).

Intersecting with these observations are new data that connect both ADAP and SKAP-55 in signaling events distinct from adhesion. ADAP has been reported to activate nuclear factor- κ B (NF κ B) signaling, an event not needed for ADAP–SKAP-55-dependent regulation of cell adhesion [72]. Similarly, SKAP-55 binds to the activator of Ras, RasGRP1, [73,74] and can negatively regulate the activation of the Ras-extracellular-regulated kinase (ERK) pathway in T cells [73,74]. Given that Ras and Rap1 can each influence the functions of the other in many cell types [75], whether an effect on Ras influences its ability to modulate Rap1 function needs further clarification. Overall, the ability of these proteins to mediate events, in addition to adhesion, provides a potential mechanism by which adhesion could directly influence antigen-receptor signaling in immune cells.

Concluding remarks

The haematopoietic-specific adaptors SLP-76, ADAP and SKAP-55 have recently been identified as key adaptors in the TCR ‘inside-out’ pathway that upregulates integrin activation and T-cell adhesion. ADAP controls SKAP-55 protein stability and turnover, whereas SKAP-55 does not exert an obvious effect on ADAP expression. The comparative phenotypes of the ADAP-deficient and SKAP-55-deficient mice are most consistent with a model in which SKAP-55 is the effector of the ADAP–SKAP-55 module. However, the requirement for the ADAP–SKAP-55 module varies with the strength of the TCR signal and points to the existence of an alternate pathway by which more potent TCR signaling can engage LFA-1 adhesion. The down-stream molecules of the ADAP–SKAP-55 module might involve the Rap1–Riam or Rap1–RapL complex. ADAP and SKAP-55 might participate in other signaling pathways that induce NF- κ B activation, but inhibit ERK activation. The BCR on B cells uses a structurally related SKAP-55R to upregulate Rap1 or Rap2 in adhesion. Future studies are needed to unravel the similarities and differences related to the

mechanism by which the TCR and BCR complexes couple the SKAP-55 and SKAP-55R adaptors with the upregulation of integrin adhesion.

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Figure 1.

Roles of integrin-mediated adhesion in T-cell function. **(a)** Integrin-mediated T-cell transmigration through blood vessels. Selectins expressed on T cells weakly interact with the vascular endothelium to induce rolling and tethering of T cells. Chemokines or TCR signaling activate LFA-1 and $\alpha 4$ integrins. This increases the avidity of integrins to enable them to firmly bind to the ligands, ICAMs or VCAMs, such that T cells are arrested at this stage. These T cells then migrate along the blood vessel or transmigrate through the endothelium, and are mediated via adhesion molecules, such as CD31 [also known as PECAM-1 (platelet endothelial cell-adhesion molecule-1)], CD44 (homing function and Indian-blood-group system) and JAM1 (junctional adhesion-molecule 1). **(b)** Integrins mediate T-cell-antigen-presenting cell (APC) conjugate formation and the immunological-synapse (IS) formation. In antigen presentation, initially weak contacts mediated by integrins are followed by ligation of the TCR complex that induces 'inside-out' signals, which fully activate the high avidity of LFA-1. In the contact area of the T-cell-APC conjugate, receptors are rearranged into signaling clusters (termed microclusters) and eventually form mature immunological synapses, which are also termed supermolecular activation clusters (SMACs). TCR-CD3, CD4 and 8, CD28 or protein kinase C θ (PKC θ) are translocated to the center of the contact area to form the cSMAC, while a ring of LFA-1 and talin forms the pSMAC.

Figure 2.

TCR-mediated 'inside-out' signaling events that lead to increased LFA-1 adhesion. After TCR is linked to the peptide-MHC complex, CD4- or CD8-associated LCK activates ZAP-70, which, in turn, phosphorylates key tyrosines on LAT, leading to the recruitment of phospholipase Cγ1 (PLCγ1) and GADS that binds constitutively in a complex with SLP-76. ITK is recruited to the complex for the phosphorylation of PLCγ1 and the mobilization of intracellular calcium. The binding of the SLP-76 SH2 domain to ADAP provides a link to SKAP-55 as an effector for LFA-1 clustering. ADAP itself functions as a chaperone for SKAP-55 and SKAP-55R degradation and binds to VASP and dynein, which could regulate the actin cytoskeleton (not shown). Further downstream, SKAP-55 might interact with Rap1-binding protein Riam or RapL and promote Rap1 translocation to the plasma membrane to regulate LFA-1 clustering. In B cells, BCR engagement might initiate the activation of SYK kinase that, in turn, phosphorylates the SLP-76 homolog SLP-65. The manner by which SLP-65 recruits SKAP-55R and further downstream molecules to activate integrins is not yet known.

Figure 3.

Structures of ADAP, SKAP-55 and SKAP-55R and their binding partners. The molecular-domain structure of ADAP, SKAP-55 and SKAP-55R. ADAP has a proline-rich domain, multiple tyrosine sites, two putative nuclear localization sites, two SH3 domains and an EVH1-domain binding site. SKAP-55 and SKAP-55R contain a PH domain, a C-terminal SH3 domain and three tyrosine-based residues, whereas the N-terminal region in SKAP-55R also has a well-defined coiled-coil domain. Abbreviations: EVH1, Ena (enabled)-VASP (vasodilator-stimulated phospho protein) homology-1; NLS, nuclear localization sites; PH, pleckstrin homology domain; PPP, proline-rich region; SH3, Src homology 3 domain; YYY, tyrosine phosphorylation motifs.

Table 1
Summary of the binding partners, expression patterns and the role of ADAP, SKAP-55 and SKAP-55R in the regulation of cell adhesion^a

	Binding partners	Expression patterns	Regulation of adhesion	Refs
ADAP	SLP-76, Fyn, c-Src, SKAP-55, SKAP-55R, Ena and VASP, dynein, Cerna1, YopH, mAbp1, SHPS1, phospholipids	T cell, platelet, mast cell, macrophage, NK, osteoclast precursor (not in B cell)	T-cell, platelet and macrophage adhesion	[21,27,30-32,34-37,39-42,50,54,56,58,59,72]
SKAP-55	ADAP, Fyn, Riam, RapL, RasGRP	T cell, mast cell, macrophage (not in B cell)	T-cell adhesion	[30,31,32,36,45-49,66,73,74]
SKAP-55R	ADAP, YopH, SHPS1	T cell, B cell, mast cell, macrophage, platelet	B-cell and macrophage adhesion	[33,51,56]

^aAbbreviations: mAbp, mammalian actin-binding protein 1; SHPS1: SH2-domain bearing protein tyrosine phosphatase (SHP) substrate-1.