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Emerging Roles of ADAP, SKAP55, and SKAP-HOM for Integrin and NF-κB Signaling in T cells

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Abstract

Adapter proteins are scaffolding proteins that lack enzymatic or transcriptional activity. They play a crucial role in the organization of signalosomes, which are molecular complexes involved in signal transduction. Here, we review some of the recent findings regarding the three cytosolic adapter proteins ADAP, SKAP55, and SKAP-HOM with respect to their role in T-cell adhesion, migration and proliferation.

Keywords: ADAP; SKAP55; SKAP-HOM; Integrins; Inside-out-signaling; Outside-in-signaling; NF-κB

Abbreviations: ADAP: Adhesion and Degranulation-promoting Adapter Protein; APCs: Antigen-Presenting Cells; CARMA1: Caspase Recruitment domain Membrane-Associated guanylate kinase protein 1; DCs: Dendritic Cells; Gads: Grb2-related adapter protein downstream of Shc; JNK: c-Jun kinase; LAT: Linker for Activation of T cells; LFA-1: Leukocyte Functional Antigen-1; MAPK: Mitogen-Activated Protein Kinases; Mst1: Mammalian Ste20-like kinase; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cell; PH: Pleckstrin Homology; PIP_3: Phosphoinositol-(3,4,5)-triphosphate; PKCθ: Protein Kinase C theta; PLCγ1: Phospholipase Cγ1; RAPL: Regulator for cell Adhesion and Polarization enriched in Lymphoid tissues; RIAM: Rap1 Interacting Adapter Molecule; SKAP55: Src Kinase Associated Protein of 55 kDa; SKAP-HOM: SKAP-Homologue; SLOs: Secondary Lymphoid Organs; SLP-76: SH2-domain-containing Leukocyte Protein of 76 kDa; TAK1: Transforming growth factor-β-activated protein kinase; TCR: T-cell receptor; VLA-4: Very Late Antigen-4; ZAP-70: Zeta-chain-Associated Protein kinase 70

Introduction

For the initiation of an adaptive immune response, T cells circulating in the blood stream need to adhere to the endothelial vessel walls before they migrate into secondary lymphoid organs (SLOs). Within the SLOs, T cells encounter antigen-presenting cells (APCs) displaying foreign peptide-MHC complex that are recognized by T cell receptors (TCR) leading to the formation of stable T cell-APC contacts. The interactions of T cells with endothelial cells or APCs are critically dependent on integrins, e.g. the β1-integrin Very Late Antigen-4 (VLA-4) and the β2-integrin Leukocyte Functional Antigen-1 (LFA-1) [1-3]. Given the importance of integrin-mediated interactions of T cells with other cell types, there has been major interest in defining the intracellular pathways responsible for T-cell adhesion and migration.

On resting T cells, integrins are expressed in inactive states that adopt a low-affinity conformation for their ligands. Members of the intercellular adhesion family (ICAM 1-5) are the physiological ligands of LFA-1 whereas VCAM (vascular cell adhesion molecule for VLA-4) and fibronectin are the ligands for β1-integrins [1-3].

Triggering of the TCR by peptide-MHC complex or stimulation of chemokine receptors (e.g. CCR7 by CCL21 or CXCR4 by CXCL12) induces a conformational change of the integrins that increases their ligand binding (affinity regulation) and subsequently mediates clustering of integrins at the cell surface (avidity regulation). The intracellular molecular events leading to integrin activation have collectively been termed "inside-out signaling". Conversely, ligand bound integrins transmit a signal to the T cell ("outside-in signaling") and thereby promote activation, proliferation, and migration of T cells [1-3].

Because integrin-mediated signaling controls T-cell function, defining the intracellular pathways required for TCR-mediated inside-out/outside-in signaling events are of major interest. After ligation of the TCR by peptide-MHC complex, the Src family protein tyrosine kinases Fyn and Lck phosphorylate tyrosines within the immunoreceptor tyrosine-based activation motifs (ITAMs) of the cytoplasmic domains of the CD3 subunits of the TCR complex. In their phosphorylated state, the ITAMs recruit the Syk-related Zeta-chain-associated protein kinase 70 (ZAP-70), which is subsequently phosphorylated and activated by Src kinases. Together, activated Src and Syk-kinases phosphorylate multiple intracellular molecules including adapter proteins and thereby couple the TCR to downstream signaling events that culminate in cellular activation of T cells.

Adapter proteins are molecules that lack enzymatic or transcriptional activity and are composed of protein-protein and protein-lipid interaction domains. They mediate the formation of macromolecular complexes (signalsomes) that are responsible for signal transduction. Adapter proteins are divided into two groups: transmembrane adapter proteins and cytosolic adapter proteins [4-6].

The phosphorylation of the transmembrane adapter protein Linker for activation of T cells (LAT) by ZAP-70 induces the recruitment of a

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complex consisting of the two cytosolic adapter proteins Grb2-related adapter downstream of Shc (Gads) and SH2-domain-containing leukocyte protein of 76 kDa (SLP-76). The formation of the LAT/Gads/SLP-76 signalosome provides a platform for the phosphorylation/activation of various signaling molecules including Phospholipase Cγ1 (PLCγ1), Protein Kinase C theta (PKCθ), Mitogen-activated protein kinases ((MAPK), ERK1/2, p38, and c-Jun kinase (JNK)), the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cell), the small GTPase Rap1, and other adapter proteins crucial for integrin activation and proliferation [7-9].

The GTPase Rap1 has been implicated as a regulator for TCR-mediated integrin activation [10]. Recently the two Rap1 effector proteins Rap1 Interacting Adapter Molecule (RIAM) and Regulator for cell Adhesion and Polarization enriched in Lymphoid tissues (RAPL) have been shown to preferentially interact with the activated (GTP-bound) form of Rap1 [11,12]. In addition to Rap1 binding to RAPL, the mammalian Ste20-like kinase Mst1 has been identified as a binding partner of RAPL [13]. RAPL and Mst1-deficient T cells have severe defects in TCR-mediated adhesion of β1-and β2-integrins and in T-cell interaction with APCs [11,13,14]. In addition, RAPL/Mst1-deficient lymphocytes show impaired chemokine-mediated LFA-1 adhesion, homing into SLOs, and intranodal migration *in vivo* [14-16]. Gain-of-function and loss-of-function studies in Jurkat T cells showed that RIAM is involved in TCR-induced activation of β1-and β2-integrins and T-cell adhesion [12]. So far it is not known whether RIAM regulates integrin-mediated lymphocyte adhesion and migration triggered by chemokines.

In this review, we summarize recent advancements that put forward our understanding of how the cytosolic adapter proteins ADAP, SKAP55, and SKAP-HOM are connected with Rap1, RAPL/Mst1 and RIAM to regulate inside-out/outside-in signaling of integrins. Besides the role of ADAP in integrin activation, recent studies indicate that this cytosolic adapter protein is crucial for the activation of NF-κB in T cells [17,18].

Cellular Expression and Structural Features of ADAP, SKAP55, and SKAP-HOM

Adhesion and Degranulation-promoting adapter protein (ADAP), also known as FYB (Fyn-T binding protein) or SLAP-130 (SLP-76 associated phosphoprotein of 130 kDa) was identified as an interaction partner of the Src kinase Fyn and the cytosolic adapter protein SLP-76 [19-22]. ADAP is expressed in thymocytes, peripheral T cells and in a variety of other hematopoietic cells [7,19,20]. Src kinase associated protein of 55 kDa (SKAP55), also termed Skap1, was identified as a substrate of the Src kinase Fyn [23] and is exclusively expressed in T cells [23-25]. Similar to SKAP55, SKAP-Homologue (HOM) (also known as SKAP55-R or Skap2) was also identified as substrate of the Src kinase Fyn [26,27]. However, in contrast to SKAP55, SKAP-HOM is expressed in T cells and in a variety of other cell types [7,24].

Depletion experiments revealed that ~70% of the endogenously expressed ADAP is constitutively associated with SKAP55 whereas all endogenous expressed SKAP55 is associated with ADAP in Jurkat T cells [28]. Huang et al. observed that the SKAP55 protein expression, but not the mRNA, is undetectable in ADAP-deficient Jurkat T cells [29]. Similarly, T cells obtained from ADAP-deficient mice not only revealed a loss of SKAP55 but also of SKAP-HOM. This indicates that the constitutive association between ADAP and SKAP55/SKAP-HOM is required for stable expression of both adapter proteins [24,25,30]. Conversely, neither loss of SKAP55 nor SKAP-HOM affected the stability of ADAP [24,31]. Recent data indicate that SKAP55 is subject to proteasome- and caspase-driven proteolysis. In addition, Huang et al. [29] observed that ADAP induces SKAP55 redistribution from a detergent-soluble to a detergent-insoluble fraction to protect SKAP55 from degradation. These data suggest that ADAP-dependent targeting of SKAP55 to a different subcellular compartment that is less accessible for the proteolytic machinery [29]. Clearly, further investigations are required to address how ADAP facilitates SKAP55 stabilization.

ADAP, SKAP55 and SKAPHOM are characterized by several folded adaptor domains, flanked or interspersed by intrinsically unstructured regions. The latter comprise interaction motifs that in some cases depend on the post-translational modification of individual residues. For example, ADAP contains several tyrosine residues that can mediate interactions with the SH2 domains of SLP-76 (Y^{595}DDV and Y^{651}DDV), the Src kinase Fyn (Y^{625}DGI), and the SH2 domain of the cytoskeletal adaptor protein Nck (Y^{595}DDV) [19,21,22,32]. The direct link of ADAP to the important regulator of the actin cytoskeleton was independently confirmed by two groups [33,34], and one of which also showed that the ADAP-Nck interaction fosters the SLP-76 interaction with the actin nucleation Wiskott-Aldrich syndrome protein (WASp) [34].

While a functional role of ADAP tyrosine phosphorylation has been established for SLP-76, Fyn and Nck [19-22,32,33], additional tyrosines, namely those at position 559, 571, 755, 771 and 780 are reported to be phosphorylated (http://www.phosphosite.org/homeAction.do) and were reproduced in our *in vitro* kinase assay. Tyrosine-to-phenylalanine mutants of these sites exert moderate to significant effects on either adhesion or migration of T cells [22]. Since their mechanistic role in T cell signaling is less well understood they were left out in Figure 1 for clarity. In addition, it is noteworthy that a proteomic screen with phosphorylated peptides comprising the Y^{595}DDV, the Y^{625}DGI and the Y^{771}DDI motifs identifies a defined set of SH2 domain containing proteins that bind to all three motifs and include SLP-76 and Nck [35]. One interesting scenario is that ADAP presents a phosphorylation hub, where several SH2 domain containing proteins transiently colocalize, depending on relative concentration, subcellular localization, and additional geometric constraints as they are for example imposed by a biological membrane. Whether the binding of Fyn to ADAP creates a feed-back-loop for the phosphorylation of additional, less accessible tyrosine sites in ADAP or its directly bound interaction partners are an interesting question. Two possible candidates for such tyrosine residues are Y571 and Y757 in ADAP, which are positioned within the domain borders of the N- or the C-terminal helically extended Src homology 3 (hSH3) domains, respectively. Fyn-SH2 binding to the phosphorylated Y^{625}DGI site of ADAP would enhance the colocalization of the two proteins, thereby enabling the phosphorylation of the less accessible tyrosine motifs at the C-termini of the hSH3 domains. Such hierarchical tyrosine phosphorylation by Src family kinases has been demonstrated for the adaptor protein and guanine nucleotide exchange factor Vav. In this case binding of the Src kinase SH2 domain to intrinsically more unstructured phosphorylated tyrosine sites precedes the phosphorylation of a tyrosine residue in a short autoinhibitory helix at the beginning of the Dbl homology domain of Vav, thereby imposing more strict requirements for the full activation of the protein [36].

The direct and reversible interaction of ADAP with lipid membranes is mediated by its two hSH3 domains [37]. In this unusual variant of the SH3 fold an N-terminal α-helix packs against the β-sheet of the canonical SH3 domain structure. Thereby the stability of the domain is enhanced and the helix displays several positively charged side chains that likely favor membrane binding [37,38]. While the

more N-terminal hSH3 domain has a low intrinsic membrane binding affinity that might be modulated by reversible lipid modification of two neighboring cysteines, the C-terminal hSH3 domain preferably interacts with highly negatively charged phosphoinositides such as phosphoinositol-(4,5)-bisphosphate (PIP_2) or phosphoinositol-(3,4,5)-triphosphate (PIP_3). However the latter interaction does not seem to rely on the recognition of a specific phosphoinositide head groups, but rather is based on charge density of the respective membrane surface [37,38].

In between the two hSH3 domains one Ena/vasodilator-stimulated phosphoprotein (VASP) homology 1 (EVH1)-binding site has been identified (Figure 1) [39]. Before the N-terminal hSH3 domain is a proline-rich region that specifically binds to the SH3 domain of SKAP55 (discussed below; Figure 1) [19-22,40]. Recently, two inducible binding sites for CARMA1 (caspase recruitment domain (CARD) membrane-associated guanylate kinase (MAGUK) protein 1) and TAK1 (transforming growth factor-β (TGFβ)-activated protein kinase) have been identified in ADAP, all of which are critically involved in the activation NF-κB and cell cycle progression (discussed below) [17,41] (Figure 1).

SKAP55 contains a putative N-terminal dimerization domain followed by a ~ 60 amino acid (aa) linker, a Pleckstrin Homology (PH) domain, an interdomain that carries three tyrosine-based signaling motifs, and a C-terminal SH3 domain [23,25] (Figure 1). SKAP-HOM and SKAP55 share 44% identity on the protein level and possess a similar overall structure with the greatest conservation in the PH and the SH3 domain [26,27,42]. One difference between SKAP55 and SKAP-HOM relates to the fact that the SKAP-HOM interdomain contains only two tyrosine-based signaling motifs whereas SKAP55 possess 3 of those signaling motifs (Figure 1) [26,27]. Whether the N-terminal region of SKAP55 also contains a coiled-coil region as seen in the structure of SKAP-HOM needs to be investigated; however the observation that the fragment of SKAP55 that forms homodimers in SKAP-HOM [42] is dimeric (Figure 1) strongly suggests structural homology (Meineke/Freund, unpublished data).

Swanson et al. have recently reported that the isolated PH domain of SKAP-HOM binds the phospholipids (PIP_3) to an arginine residue at position 140 (R140) [42]. In contrast to the PIP_3 binding capacity of the isolated PH domain, the binding of PIP_3 to a SKAP-HOM fragments consisting of the N-terminal CC and PH domains (CC-PH fragment) is dramatically reduced. Structural analysis of the CC-PH fragment revealed that the phospholipid-binding pocket is perturbed through interaction of the CC domain with the PH domain. The inhibitory interaction between the CC and the PH domains suggests that SKAP-HOM exists in two states with distinct PIP_3-binding abilities: a closed, inactive state, and an open conformation capable of lipid binding. PIP_3 as the lipid product of the phosphatidylinositol 3-kinase (PI3K) is required for plasma membrane recruitment of PH domain containing proteins [43]. However, lipid binding is probably not the only factor driving membrane localization, since a SKAP-HOM construct missing the entire PH domain is still localized to the membrane ruffles [42]. Presumably, a protein-protein interaction between the autoinhibitory N-terminus and an elusive binding partner acts in conjunction with the lipid binding event, thereby creating a coincident detector as it has been observed in other cytoplasmic adaptor proteins such as WASP [44]. A strong candidate for this protein interaction partner is RAPL and there is some experimental evidence that the coiled-coil domain of RAPL binds to the SKAP55 N-terminus [45]. It now has to be evaluated whether the individual binding events are indeed interdependent and *in vitro* as well as *in vivo* approaches should be invoked to answer this mechanistically important question.

The high amino acid identity near the critical R140 residue within the SKAP-HOM PH domain lead to the identification of an arginine at position 131 (R131) within the PH domain of SKAP55 that may have the same lipid-binding properties for PIP_3 as SKAP-HOM [46] (Figure 1). Inhibition of the PI3K reduced the recruitment of SKAP55 to the plasma membrane in TCR-stimulated primary mouse T cells [47]. Mutation of the arginine at position 131 to methionine (R131M) within the PH domain abolished plasma membrane recruitment of SKAP55 [46,47]. These data suggests that similar to the PH domain of SKAP-HOM, the PH domain of SKAP55 binds PIP_3 and these lipid-binding properties are required for PI3K-dependent plasma membrane targeting of SKAP55.

Both, SKAP55 and SKAP-HOM have been reported to constitutively interact with ADAP in T cells [24,27,28]. The binding between ADAP and SKAP55/SKAP-HOM involves the proline-rich region of ADAP, which interacts with the SH3 domain of SKAP55/SKAP-HOM [27,28]. A 24-aa segment between aa 340–364 within the proline-rich region of ADAP was identified to mediate an interaction with the SH3 domain of SKAP55 and with the tryptophan at position 333 being critically involved in binding, as it is expected for a canonical SH3 domain interaction [25,28,30] (Figure 1).

ADAP-, SKAP55-, and SKAP-HOM-deficient Mice

As described above, at least with regard to the T-cell compartment, the ADAP-deficient mouse is a triple knockout mouse that lacks the expression of ADAP, SKAP55, and SKAP-HOM [25]. ADAP-deficient mice have been generated by two independent groups [48,49]. Analysis of the T-cell compartment revealed that ADAP-deficient mice have a reduced total cell number of peripheral T cells, but the percentages of $CD4^+$ and $CD8^+$ T cells compared to wild type T cells are not affected

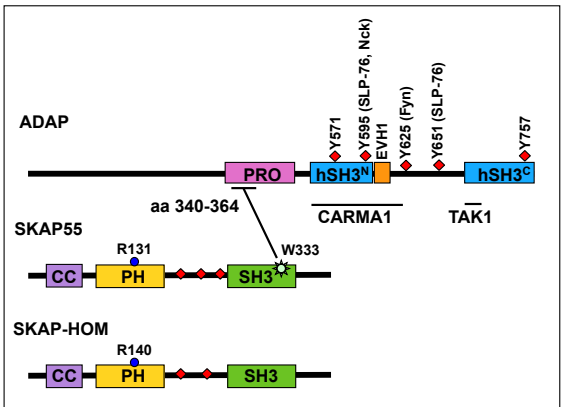

Figure 1: Structure of ADAP, SKAP55, and SKAP-HOM. Amino acid (aa) numbering are given for human ADAP isoform 2 (NM1999335.3), SKAP55 (Y11215.1) and SKAP-HOM (AJ004886.1). ADAP has a N-terminal proline-rich domain (PRO), multiple tyrosine-based signaling motifs, two helical SH3 domains (hSH3), an Ena-VASP homology 1 (EVH1)-binding site, and binding sites for CARMA1 (caspase recruitment domain (CARD) membrane-associated guanylate kinase (MAGUK) protein 1) as well as for TAK1 (transforming growth factor-β (TGFβ)-activated protein kinase). The amino acids stretch 340-364 within the proline-rich domain of ADAP interacts with the tryptophan (W) at position 333 within the SH3 domain of SKAP55. SKAP55 and SKAP-HOM, both contain a central PH domain and C-terminal SH3 domain. In contrast to the three tyrosine-based signaling motifs SKAP55 (indicated as red squares), SKAP-HOM possesses only two of these motifs. Both SKAP-HOM and SKAP55 contain a coiled-coil region (CC) important for dimerization and an arginine (R) at position 140/131 within the PH domain that binds probably to PIP_3.

[49]. Further investigations employing various TCR transgenic mouse models indicated that ADAP is required for positive and negative selection of thymocytes [50]. In addition to the defective T-cell development, peripheral T-cell functions were also impaired in ADAP-deficient T cells. Thus, peripheral ADAP-deficient T cells showed reduced proliferation, impaired production of IL-2 and IFNγ and diminished CD69/CD25 upregulation upon CD3/CD28 stimulation [48,49]. Biochemical analysis of TCR/CD28-mediated activation of the NF-κB and the MAPK JNK are impaired, whereas the activation of MAPK ERK1/2 and p38 are not affected in ADAP-deficient T cells [17,40,48,49,51 and Kliche unpublished data]. However, ADAP-deficient T cells display reduced interactions with APCs, altered TCR-mediated adhesion for both the β1- and β2-integrins, and diminished LFA-1 clustering [48,49].

SKAP55-deficient mice are born healthy and exhibit no major alteration in T-cell maturation. Comparable, but not as pronounced as for ADAP-deficient animals, SKAP55-deficient T cells show reduced proliferation, impaired IL-2 and IFNγ production, and attenuated CD69/CD25 upregulation upon TCR-stimulation [31]. Moreover, loss of SKAP55 reduces TCR-mediated β1- and β2-integrin adhesion and LFA-1 clustering to a similar extends as deficiency of ADAP. In addition, SKAP55-deficient T cells show reduced contact times with superantigen-loaded dendritic cells [31]. The short lived contacts are paralleled by reduced proliferation and IL-2 production of SKAP55-deficient T cells, indicating that an inefficient interaction with APCs accounts for the impaired T-cell activation and proliferation.

In contrast to SKAP55-deficient animals, SKAP-HOM-deficient mice display normal T-cell development and their T cells do not show any apparent functional defects [24]. This indicates that SKAP55 compensates for the loss of SKAP-HOM. The similar functional properties of SKAP55 and ADAP in T cells and the biochemical work described above show that (1) SKAP55 and ADAP constitutively interact with each other; (2) all SKAP55 molecules expressed in T cells are associated with ADAP; and (3) the expression of ADAP is required for stable expression of SKAP55 in T cells. This suggests that ADAP and SKAP55 form a functional signaling unit, the ADAP/SKAP55-module, that regulates integrin activation in T cells.

In addition to its central role in TCR-mediated adhesion and T-cell interaction with APCs, we have previously shown that the ADAP/SKAP55-moduleis also required for chemokine-mediated affinity/avidity regulation of LFA-1, T-cell adhesion and migration *in vitro*. Moreover, the loss of the module delays homing of T cells into SLOs *in vivo,* probably due to a defect in chemokine-mediated adhesion [52]. By using intravital microscopy to analyze the interaction of ADAP-deficient T cells with the high endothelial vessel walls closed to the peripheral lymph node (LN), we observed that ADAP-deficient T cells have reduced contact times with the endothelial layer (Degen/Reichardt/Kliche unpublished data). In addition, intravital microscopy within the LN parenchyma showed that loss of the ADAP/SKAP55-modulealso attenuated the random walk of T cells [52]. The delayed homing into the LN, the reduced motility within the LN and the defective interaction with APC of ADAP-deficient T cells might also affect their efficiency to encounter dendritic cells for peptide-MHC to initiate an adaptive immune response. In two transplantation models, ADAP-deficient mice showed reduced rejection of heart graft or of intestinal allografts [53,54]. These graft protections have been accompanied by reduced infiltration, proliferation, and activation of T cells in the allografts [53,54].

Surprisingly, in contrast to loss of ADAP, loss of either SKAP55 or SKAP-HOM is dispensable for both CXCL12 and CCL21 induced T-cell migration *in vitro* [52,55]. These data could suggest that with regard to chemokine stimulation SKAP55 and SKAP-HOM have redundant functions in T cells and it has now to be investigated whether T cells from SKAP55/HOM double knockout mice show impaired chemokine-mediated integrin activation and T-cell homing *in vivo*.

The Role of the ADAP/SKAP55-module in Chemokine-mediated Integrin Activation

The similar function of RIAM and ADAP/SKAP55-deficient T cells for integrin activation indicated a possible connection of the ADAP/SKAP55-module to this Rap1 effector protein. In co-immunoprecipitations studies, we found RIAM to be constitutively associated with the ADAP/SKAP55-module and SKAP55 is the binding partner of RIAM [56]. In addition, others have shown that SKAP55 directly interacts with RAPL [45]. During our attempts to assess the molecular mechanisms of how the ADAP/SKAP55-module regulates chemokine-mediated affinity/avidity regulation of LFA-1, we identified two independent molecular complexes of the ADAP/SKAP55-module. One complex contains RIAM, Mst1, Rap1 and the two FERM-domain containing proteins Talin and Kindlin-3 (Figure 2). The second complex consists of RAPL, Mst1 and Rap1. Both the RAPL/Mst1/Rap1 and the RIAM/Rap1/Mst1/Kindlin-3/Talin complexes require the presence of the ADAP/SKAP55-module for binding to LFA-1 upon chemokine stimulation [52]. Each of the two complexes appears to independently interact with the α-chain (CD11a) of LFA-1 probably via RAPL [11] and the β-chain (CD18) of LFA-1 via Kindlin-3 [57], respectively [52] (Figure 2). These findings indicate that the associations of RAPL and RIAM with the ADAP/SKAP55-module are crucial for chemokine-mediated LFA-1 activation. Currently it is not clear whether the ADAP/SKAP55-modules concomitantly regulates inside-out signaling leading to the activation of LFA-1 or whether one of the pools regulates inside-out signaling whereas the other pool regulates outside-in signaling.

The Role of the ADAP/SKAP55-module in TCR-mediated Integrin Activation

Data from two previous studies had indicated that the two of the ADAP/SKAP55-modules not only regulate chemokine signaling but either linked to RIAM or RAPL, are also involved in TCR-mediated integrin activation (Figure 2). We have shown that disruption of the interaction between SKAP55 and RIAM diminishes TCR-mediated adhesion and T-cell interaction with APCs [25,56]. Furthermore, we showed that loss of the ADAP/SKAP55 proteins attenuated plasma membrane targeting of both RIAM and its upstream effector Rap1 [56]. In addition, others have demonstrated that disruption of the interaction between SKAP55 and RAPL (or loss SKAP55) impairs the binding of RAPL/Rap1 to LFA-1 and subsequently reduces TCR-mediated adhesion and T-cell-APC interaction [45]. Since we found a constitutive interaction of the ADAP/SKAP55-module with RAPL/Mst1 or RIAM/Mst1/Kindlin-3 in resting primary human T cells, we assume that both complexes are associated with LFA-1 upon TCR stimulation [45] (Figure 2). Collectively these data show that the associations of RAPL and RIAM with the ADAP/SKAP55-module are crucial for T-cell adhesion and T-cell interaction with APCs.

Raab et al. [47] showed that SKAP55 is required for RAPL translocation to the plasma membrane. This TCR-induced membrane recruitment of SKAP55/RAPL to LFA-1 is dependent on the PH domain of SKAP55 and an intact PI3K pathway [47]. After TCR-stimulation the two tyrosines residues Y595 and Y651 in ADAP are phosphorylated

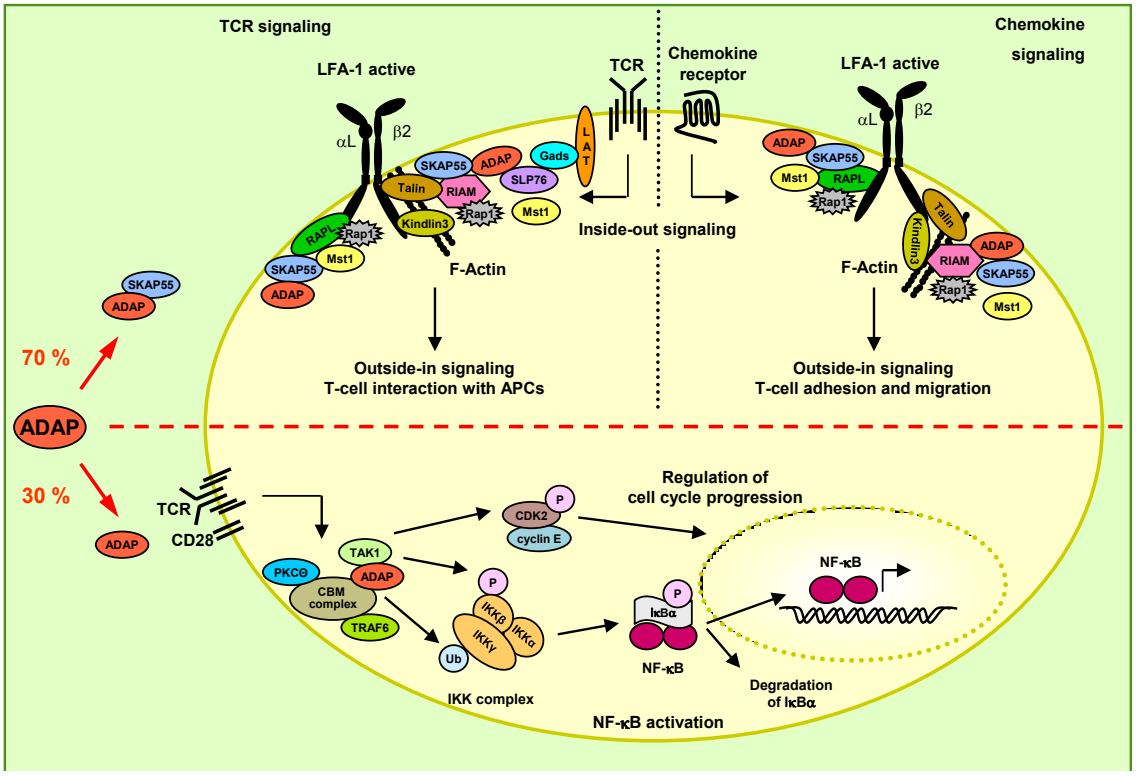

Figure 2: Schematic model of how the two distinct pools of ADAP coordinate NF-κB activation or integrin activation. The pool of ADAP associated with SKAP55 (app. 70%) regulates integrin function. In response to chemokine receptor stimulation, the ADAP/SKAP55/RIAM/Mst1/Kindlin-3/Rap1 and the ADAP/SKAP55/RAPL/Mst1/Rap1 appear to independently interact with the α-chain (CD11a) probably via RAPL and the β-chain (CD18) of LFA-1 via Kindlin-3 to facilitate LFA-1 inside-out/outside-in signaling required for T-cell migration. The interaction between the Ferm-domain containing protein Talin with the ADAP/SKAP55/RIAM/Mst1/Kindlin-3/Rap1 may provide a further link to F-Actin. Similar to chemokine stimulation, upon TCR triggering, the two distinct ADAP/SKAP55/RIAM/Mst1/Kindlin-3/Rap1 and the ADAP/SKAP55/RAPL/Mst1/Rap1 are recruited either via the inducible association between SLP-76 and ADAP or via the PIP_3-binding property of the PH domain of SKAP55 to the plasma membrane in close proximity to the integrin LFA-1 to promote T-cell interaction with APCs. The pool of ADAP not associated with SKAP55 (app. 30%) is most likely involved in NF-κB activation and cell cycle progression upon TCR/CD28 stimulation. In this scenario ADAP is required for the assembly of the PKCθ/CBM/TRAF6/ADAP/TAK1 signalosome. The presence of ADAP within this signalosome controls IKK complex activation by polyubiquitination of IKKγ by TRAF6 and IKKβ phosphorylation via TAK1. The activated IKK phosphorylates IκBα thereby facilitating the degradation of this inhibitory subunit of the NF-κB transcription factor. NF-κB now translocatesto the nucleus controls NF-κB-dependent gene expression. Besides the role of ADAP for NF-κB activation, the interaction of ADAP with CARMA1 and TAK1 regulates cyclin E and Cdk2 expression. Both the induction of cyclin E and Cdk2 are critically involved at the late G1 restriction point of the cell cycle.

and function as binding site for the SH2 domain of SLP-76 [19-21,58] (Figure1). Mutation of these two tyrosines abrogated TCR-mediated adhesion and T-cell interaction with APCs [58]. Hence, there is little known about the mechanism how the ADAP/SKAP55/Mst1/RIAM/Kindlin-3 or the ADAP/SKAP55/RAPL/Mst1 complexes are recruited to the plasma membrane either via SLP-76 within the LAT/Gads/SLP-76 signalosome or via the PH domain of SKAP55.

ADAP and NF-κB Signaling in T cells

Besides the role of ADAP in integrin activation, recent work has established that ADAP is also required for activation of the transcription factor NF-κB [17,51]. Thus, Burbach et al. identified two distinct pools of ADAP [30,46]. One pool associated with SKAP55 (app. 70%) regulates integrin function, while a second pool is not associated with SKAP55 (app. 30%) is involved in NF-κB activation upon TCR/CD28 stimulation [17,30,46] (Figure 2).

The signaling pathway leading to NF-κB activation is crucial for full T-cell activation and proliferation [58,59]. TCR/CD28-stimulation of T cells leads to activation of the IκB kinase (IKK) complex that contains two catalytic subunits, IKKα and IKKβ, and one regulatory subunit, IKKγ. Activated IKK phosphorylates IκBα thereby facilitating the ubiquinitation and subsequently the degradation IκBα. Loss of IκBα enables NF-κB to translocate into the nucleus and to initiate transcription NF-κB target genes. TCR/CD28-mediated activation of the Protein Kinase theta (PKCθ) phosphorylates the plasma membrane localized adapter protein CARMA1 [59,60]. Subsequently, phosphorylated CARMA1 recruits B-cell CLL-lymphoma 10 (Bcl10) and mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) to assemble the CARMA1-Bcl10-MALT1 (CBM) complex [61]. The interaction of the ubiquitin E3 ligase TRAF6 with the CBM complex is required for polyubiquitination of the IKKγ subunit. Beside the CBM/TRAF6 complex, activation of the kinase TAK1 through PKCθ leads to phosphorylation of IKKβ. Both polyubiquitination of IKKγ and TAK1-mediated phosphorylation of IKKβ are involved in IKK activation [59,60].

Biochemical analysis of ADAP-deficient T cells revealed that both polyubiquitination of IKKγ and phosphorylation of IKKβ are impaired [18]. The attenuated polyubiquitination of IKKγ and phosphorylation of IKKβ is not due alteration of the TCR/CD28-mediated activation of PKCθ and TAK1 in ADAP-deficient T cells [17,18,51]. Biochemical experiments showed that ADAP is required for the formation of the CBM complex via interaction with CARMA1 to control polyubiquitination of IKKγ and the association of ADAP with TAK1 as well as the recruitment of TAK1 to CARMA1 mediate phosphorylation

of IKKβ [17,18] (Figure 2). Two independent regions within ADAP were identified to be critical for the inducible association of ADAP with CARMA1 and ADAP with TAK1, respectively [17,18] (Figure 1). Thus, these data suggest that ADAP functions to recruit activated TAK1 to the CBM/TRAF6 complex for the assembly of the PKCθ/CBM/TRAF6/ADAP/TAK1 signalosome to promote NF-κB activation (Figure 2).

Recently, it has been shown that ADAP-deficient T cells have a block in cell cycle progression in the G1-S transition upon TCR/CD28 stimulation [41]. The temporal accumulation of cyclin and cyclin-dependent kinases (CDKs), determine the progression of T cells through the cell cycle following TCR stimulation. Cdk4, and Cdk6 are activated during the G1 phase of the cell cycle, followed by the induction of cyclin E and the subsequent activation of Cdk2 at the late G1 restriction point. Western Blot analysis revealed that cyclin E is only transiently expressed in ADAP-deficient T cells upon TCR/CD28 stimulation, likely because of enhanced ubiquitination of cyclin E [41]. The molecular mechanism of how ADAP upregulates Cyclin E ubiquitination is unclear at present.

However, both the inducible interaction of ADAP with CARMA1 and TAK1 are critical for cyclin E expression [41]. In contrast, only the association of ADAP with CARMA1 is important for Cdk2 induction [41]. Further biochemical analysis revealed that the CARMA1-binding site in ADAP is crucial for mitogen-activated protein (MAP) kinase kinase 7 (MKK7) phosphorylation and its recruitment to the PKCθ/CBM/TRAF6/ADAP/TAK1 signalosome to mediate JNK activation and moreover, for JNK-dependent Cdk2 induction [41]. These findings provide a novel molecular pathway of how ADAP regulates cell cycle progression.

Conclusion

In T cells, ADAP regulates TCR/chemokine-mediated integrin activation to promote T-cell interaction with APCs, T-cell migration and TCR/CD28-induced NF-κB signaling to promote T cell proliferation. These two functions mediated by ADAP are controlled through distinct pools that are defined by the cytosolic adapter protein SKAP55. The pool of ADAP that is not associated with SKAP55 regulates NF-κB and cell cycle progression. The pool of ADAP associated with SKAP55 regulates integrin function via the assembly of two complexes the ADAP/SKAP55/RAPL/Mst1 and the ADAP/SKAP55/RIAM/Mst1/Kindlin-3, respectively. Interestingly, the PH domain of SKAP55 seems to be crucially involved in defining these distinct sets for integrin or NF-κB activation of ADAP [56]. Moreover, the identification of Kindlin-3 as component of the ADAP/SKAP55/Mst1/RIAM/Kindlin-3 complex links the ADAP/SKAP55 module to the immunodeficiency syndrome: the "Leukocyte Adhesion Defect typ III" (LAD) [62]. Patients with this disease show recurrent infections, delayed wound healing, and bleeding [62,63]. This disease is caused by mutations in Kindlin-3 and shows defective β1, β2, and β3-integrin mediated adhesion and migration of immune cells despite normal integrin expression [62].

Integrin-mediated signaling regulated by the ADAP/SKAP55-module seems also to be required for T cell recruitment into inflamed tissues [53,54]. The prolonged allograft survival in the absence of this module was accompanied by a decreased infiltration and proliferation of activated $CD4^+$ and $CD8^+$ T cells into the graft [53,54]. Similar to the absence of the ADAP/SKAP55-module, blockade of LFA-1 by anti-LFA-1 mAbs prolonged allograft survival that accompanied by reduced T-cell proliferation and T-cell infiltration into the graft [64-66]. Thus, the ADAP/SKAP55-module might be in addition to anti-LFA-1 mAbs an interesting target to selectively block early T-cell activation events after organ transplantation and for autoimmune diseases.

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