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Review

The role of adapter proteins in T cell activation

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Abstract. Engagement of antigen receptors on lymphocytes leads to a myriad of complex signal transduction cascades. Recently, work from several laboratories has led to the identification and characterization of novel adapter molecules, proteins with no intrinsic enzymatic activity but which integrate signal transduction pathways by mediating protein-protein interactions. Interestingly, it appears that many of these adapter proteins play as critical a role as the effector enzymes themselves in both lymphocyte development and activation. This review describes some of the biochemical and molecular features of several of these newly identified hematopoietic cell-specific adapter molecules highlighting their importance in regulating (both positively and negatively) signal transduction mediated by the T cell antigen receptor.

Key words. T lymphocytes; adapter proteins; signal transduction; protein-protein interaction; lymphocyte activation.

Introduction

In recent years much has been learned about activation events which follow the interaction of lymphocytes with specific antigen. These advances related initially to the discovery of the antigen receptors themselves. However, although the structure of both the B cell antigen receptor and T cell antigen receptor (TCR) provided insights into how these molecular complexes bind to antigen, little information was gleaned from the primary receptor sequences to explain how these proteins transduce their signals. Subsequently, studies from numerous laboratories elucidated several signal transduction events which follow receptor engagement. This involved the identification of key effector enzymes (including phosphatases, kinases, and phospholipases) important for initiating proximal biochemical signals, as well as transcription factors which are required for translating these events into the activation of new genes. As this work progressed, it became increasingly clear that disparate signaling cascades needed to be integrated for lymphocyte activation. The most recent major advance in understanding lymphocyte activation has come from studies attempting to explain how these signaling events are integrated. This work has identified and characterized numerous adapter proteins, molecules with no intrinsic enzymatic properties but which function to mediate protein-protein interactions and establish larger signaling complexes. Numerous studies have demonstrated that these adapter proteins work as both positive and negative regulators of lymphocyte signal transduction and some appear to be as essential as previously described enzymes for both lymphocyte development and activation. This review focuses on a number of adapter proteins, some of which are expressed exclusively in cells of hematopoietic origin and others which are expressed more widely, and describes what is known currently about their role in the regulation of T lymphocyte activation.

The TCR and its proximal biochemical signals

The TCR is composed of a series of protein dimers

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expressed together on the cell surface [1–3]. The α and β chains arise from rearranged gene segments and are responsible for antigen recognition. The three CD3 dimers (γ/ε, δ/ε, and ζ/ζ) are non-covalently associated with the α and β chains and are responsible for transducing the antigen recognition signal into the cell. This is accomplished by the activation of src family protein tyrosine kinases (PTKs), including fyn and lck, which then phosphorylate key tyrosine residues within the CD3 chains [4–9]. The tyrosines are found in specialized domains designated immunoreceptor tyrosine-based activation motifs (ITAMs) [10]. The ITAM tyrosines, when phosphorylated, serve as docking sites for ZAP-70, a member of the syk family of PTKs [11, 12]. ZAP-70 is activated further by the src PTKs leading to subsequent phosphorylation of numerous cytosolic proteins essential for propagating the activation signal [11, 13].

One of the TCR-stimulated PTK substrates is phospholipase Cγ1 (PLCγ1), the enzyme responsible for hydrolyzing the membrane phospholipid, phosphatidylinositol 4,5 bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol 1,4,5 trisphosphate (IP_3) [14, 15]. The importance of prior PTK activation for PLCγ1 function was made clear by the observation that tyrosine phosphorylation of this enzyme is critical for its optimal activity [16–19]. Both products of PIP_2 hydrolysis are second messengers important for T cell activation. IP_3 interacts with its receptor on endoplasmic reticulum resulting in the release of calcium from this intracellular store into the cytosol. The increase in calcium is critical for activating the serine/threonine phosphatase, calcineurin, which acts on nuclear factor of activated T cells (NFAT), a transcription factor important for the activation of numerous genes required for T cell activation [20]. In unstimulated cells, phosphorylated NFAT resides in the cytosol. Following calcineurin-dependent dephosphorylation, NFAT translocates to the nucleus to serve its transcriptional activator functions. The other PIP_2 hydrolysis product, DAG, is important as a stimulator of members of the protein kinase C family of serine/threonine kinases [21]. Increasing evidence suggests that multiple members of this family play critical roles in T cell signaling [22–26].

In addition to the PLCγ1-initiated signaling cascade, TCR-stimulated PTK function is also required for activation of the ras signaling pathway [27]. Ras is a small-molecular-weight guanine-nucleotide-binding protein which resides at the plasma membrane due to post-translational fatty acid modifications. In resting cells, ras is bound to GDP; however, following TCR engagement, GDP is released allowing ras to become GTP associated and activated leading to downstream signaling events [28].

In addition to these signal transduction cascades, engagement of the TCR stimulates numerous other downstream events including modulation of lipid kinase function, internalization of cell surface receptors, and reorganization of the actin cytoskeleton. Optimal T cell activation also requires stimulation of co-receptors along with the TCR to modulate second messenger cascades [29–31]. Thus, it is obvious that for effective cellular activation, tightly regulated cross-talk among these signal transduction cascades is required. The recent description of adapter proteins and their potential role as regulators and integrators of biochemical second messenger pathways has provided considerable insight into the complex regulation of TCR-initiated signal transduction events.

Protein interaction motifs: a paradigm for adapter protein function

As noted above, following TCR engagement by antigen, the ZAP-70 PTK is recruited to the CD3 ITAMs via phosphorylated tyrosine residues. This occurs because ZAP-70 contains two specialized regions known as src homology 2 (SH2) domains that recognize phosphorylated tyrosine residues in the context of appropriate carboxyl-terminal amino acids [32, 33]. SH2 domains were initially described as regions found in all src family PTKs which share sequence homology and can interact with phosphotyrosine residues found in the correct context [34]. This discovery led to the search for other protein modules which would be able to direct intermolecular interactions. Another domain was subsequently found in all src family PTKs: SH3 or src homology 3 domains also mediate protein-protein interactions, via recognition of regions within proteins rich in proline residues [35–39]. As with SH2 domains, homology studies demonstrated that SH3 domains are found in many types of proteins and are highly conserved through phylogeny.

Once the paradigm for modular protein domains capable of mediating intermolecular interactions was established, a number of other such regions were described. In addition to SH2 domains, PTB (or phosphotyrosine-binding) domains mediate interactions based on phosphotyrosine residues [40–43]. Interestingly, the specificity for PTB domain binding resides in the amino acids amino terminal to the phosphotyrosine, as opposed to SH2 domains whose binding specificity depends on the carboxyl-terminal amino acids [44]. WW domains [named for the two tryptophans (W) located in the protein-binding site] are protein regions which bind to other proteins which either contain proline-rich re-

gions or phosphorylated serine or threonine residues [45–47]. PDZ domains are modules which interact with discrete domains that contain hydrophobic residues carboxyl-terminal of the binding site [48, 49]; and plekstrin homology, or PH domains, direct intermolecular interactions based on associations with phospholipids [50, 51]. Increasing evidence indicates that each of these modular regions plays critical roles in localizing effector molecules and creating multimeric signaling complexes. Sequence analysis of many of the key enzymes responsible for mediating signal transduction events in T cells (e.g., src and syk family PTKs and the two protein tyrosine phosphatases SHP-1 and SHP-2) revealed that these proteins contain adapter modules in addition to their enzymatic domains. This is likely important both to allow these enzymes to interact with potential substrates and because these proteins play an important role in the creation of multimeric signaling complexes. One example of this was shown in experiments demonstrating that the SH2 and SH3 domains of lck are required, in addition to the kinase domain, for optimal production of interleukin-2 in a T cell hybridoma [52]. More recently, however, other proteins have been discovered which consist only of protein interaction domains. These 'pure' adapter molecules appear to play as important a role in regulating signal transduction events as do the enzymes and other effector molecules they bridge [53]. A schematic representation of the adapter molecules discussed in this review is presented in figure 1.

The first demonstration of this critical role for molecules with adapter function came following a screen for proteins which associate with the phosphorylated tail of the epidermal growth factor (EGF) receptor. Using an expression cloning technique, Schlessinger and co-workers identified several growth-factor-receptor-binding (Grb) proteins [54]. Of these, Grb2 proved to be the most interesting. Grb2 consists of a single SH2 domain flanked by two SH3 domains. The Grb2 SH2 domain has specificity for tyrosine residues within the EGF receptor tail which are phosphorylated following binding with EGF. This allows Grb2 to translocate from the cytosol to the plasma membrane following EGF receptor stimulation and autophosphorylation. Importantly, in addition to its inducible binding to the EGF receptor, Grb2 is associated constitutively with the son of sevenless (Sos) guanine nucleotide exchange factor via proline-rich regions of Sos and the Grb2 SH3 domains [55]. Thus, following EGF receptor engagement, Sos is also brought to the membrane and placed in the vicinity to its target, ras [54, 56–60].

As would be expected, more recent studies of the EGF receptor suggest that coupling of this PTK to ras is more complex than merely inducing a direct association between the activated receptor and the Grb2/Sos complex. Thus, there is evidence indicating that there may be other molecules (for example the adapter protein Shc) which are also important in regulating EGF-receptor-mediated ras activation [61, 62]. These observations suggest that there are many levels of regulation required for the integration of biochemical signaling cascades, and underscore the important roles played by adapter proteins in this process.

SH2-domain-containing leukocyte phosphoprotein of 76 kDA (SLP-76) and linker of activation of T cells (LAT) are required for T cell development and activation

Demonstration of a paradigm by which a receptor PTK could couple to the ras signaling pathway led to investigations into the potential role of Grb2 in T cells. A number of proteins, newly phosphorylated on tyrosine residues following TCR engagement, have been shown to bind to Grb2 fusion proteins in vitro [63]. Of these, two have now been shown to play critical roles in the integration of TCR-mediated signaling cascades important for both T cell development and activation (fig. 2).

SLP-76 was isolated initially due to its ability to bind to a GST Grb2 fusion protein [64]. Primary sequence analysis of SLP-76 reveals three distinct domains, an amino-terminal acidic region containing tyrosines which are phosphorylated upon TCR engagement, a central proline-rich region able to bind SH3 domains of various molecules, and a carboxyl-terminal SH2 domain. Thus, while SLP-76 possesses no domains with known enzymatic function, this protein clearly is able to act as an adapter molecule utilizing several discrete protein interaction domains.

Unlike Grb2, SLP-76 expression is restricted to hematopoietic cells. By both protein and RNA analysis, it was shown that SLP-76 is present in thymocytes, mature T cells, macrophages, natural killer cells, and megakaryocytes, but not B cells [65]. Interestingly, it appears that SLP-76 is regulated at the protein level, as its expression varies during thymocyte development and in resting versus activated T cells. Evidence suggesting that SLP-76 may play an important role in the regulation of TCR-mediated signaling events came from transient transfection studies into the Jurkat T cell line where overexpression of SLP-76 dramatically augments TCR-induced activation of the interleukin-2 gene [66, 67]. The importance of SLP-76 in this process was corroborated further with the development of a Jurkat variant deficient in SLP-76 expression [68]. While engagement of the TCR on this cell still results in activation of src and syk family PTKs, there is a complete failure for TCR engagement to result in activation of the inter-

leukin-2 gene. Dissection of the signal transduction pathways downstream of PTK activation revealed that SLP-76 is required for the TCR to couple with tyrosine phosphorylation of phospholipase C and activation of extracellular regulated kinase (ERK) [68].

In addition to studies examining the role of SLP-76 in model cell lines, two groups have reported a striking immunologic phenotype in mice made deficient in the SLP-76 gene by targeted disruption [69, 70]. Examination of the peripheral lymphoid organs in these mice reveals the complete absence of T lymphocytes. Studies of thymocytes in the knockout mice indicate that the block in T cell development occurs at the CD3−/CD4−/CD8−/CD25+/CD44− stage, a time during T cell development when the pre-TCR must deliver a signal indicating that it has rearranged correctly and is functionally coupled to the cellular signal transduction machinery. It is particularly striking that the block in

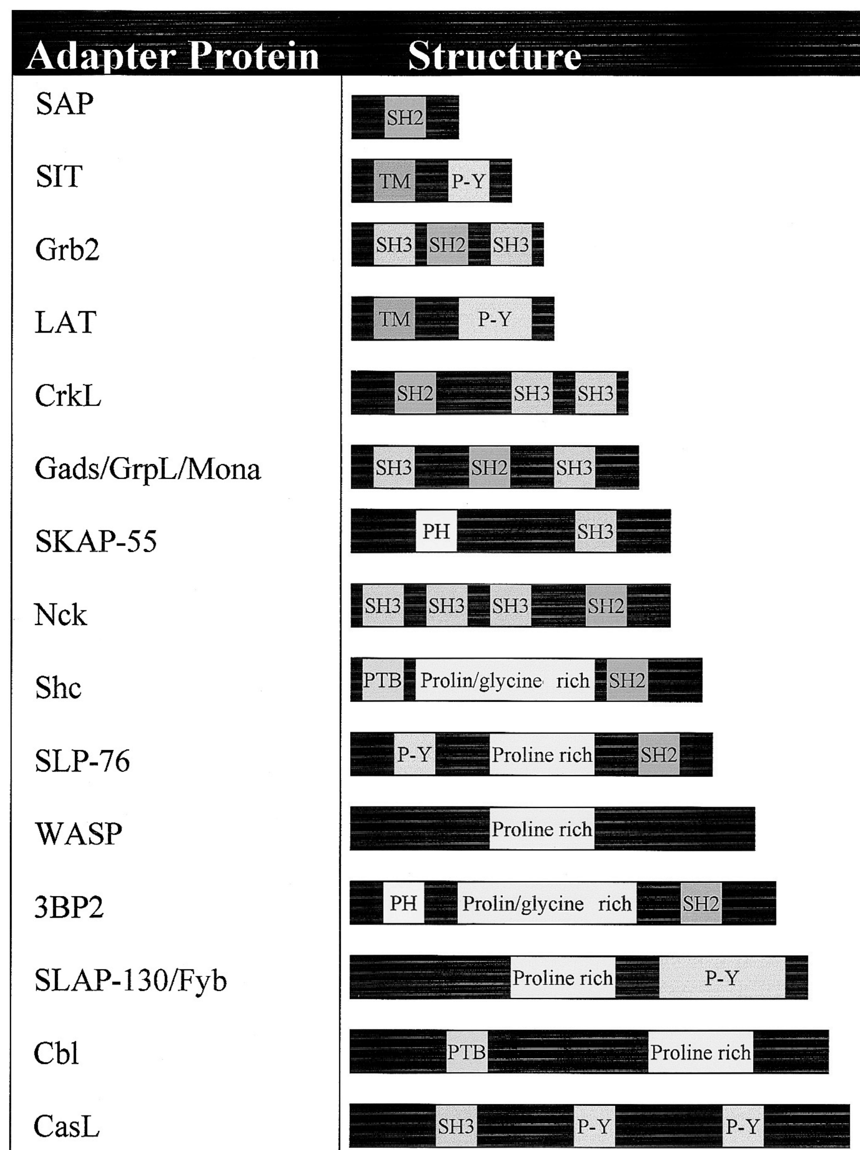

Figure 1. Adapter proteins involved in TCR signaling discussed in this review. The domain organization for each molecule is illustrated. Abbreviations: SH2, src homology 2 domain; SH3, src homology 3 domain; TM, transmembrane domain; P-Y sites, tyrosine phosphorylation site; PH, plextrin homology domain; PTB, phosphotyrosine-binding domain.

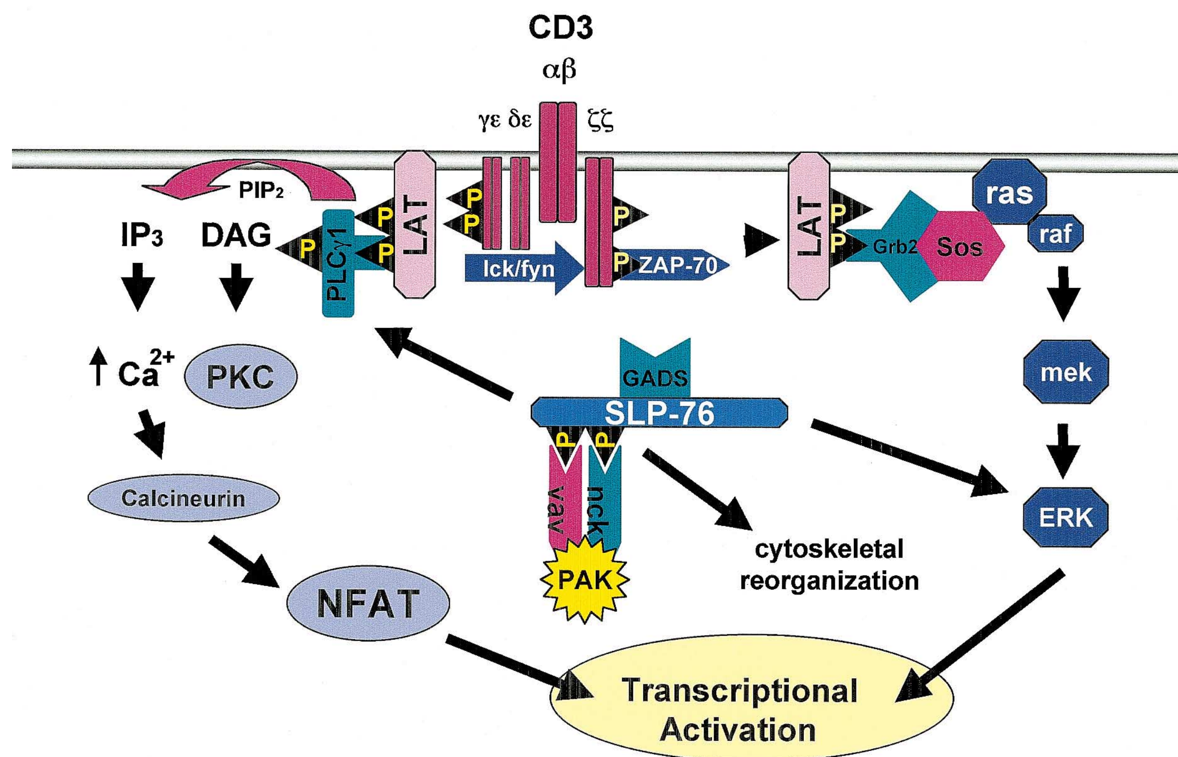

Figure 2. Model for SLP-76 and LAT function as regulators of TCR signaling. Following TCR engagement, the src PTKs, lck and fyn, are activated, leading to CD3 and ZAP-70 phosphorylation. Activated ZAP-70 phosphorylates SLP-76 and LAT initiating ras/MAPK and PLCγ1 signaling cascades. Tyrosine-phosphorylated LAT also recruits PLCγ1 to the membrane, placing it in close proximity with its substrate, PIP_2. PIP_2 is hydrolyzed to IP_3 and DAG which leads to increases in cytosolic free calcium and activation of protein kinase C (PKC). Tyrosine-phosphorylated LAT binds to the Grb2 SH2 domain which recruits Sos to the plasma membrane thus promoting ras/ERK activation. Tyrosine-phosphorylated LAT binds to the Gads SH2 domain. Since Gads constitutively associates with SLP-76, the SLP-76/Gads complex may be recruited to LAT thus promoting ras/MAPK and PLCγ1 activation. Tyrosine-phosphorylated SLP-76 also associates with Vav, promoting the formation of a SLP-76/Vav/Nck/Pak complex which may be important for the regulation of cytoskeletal rearrangements.

P denotes phosphorylated tyrosine residues.

thymocyte development is more severe in SLP-76-deficient mice than in mice lacking any single src family or syk family PTK [71].

In contrast to the severe block in T cell development, macrophages, natural killer cells, and platelets are present in SLP-76-deficient mice. Early studies of macrophage and natural killer cell function revealed that these components of the immune system are grossly intact in the SLP-76-deficient mice [72; P. S. Myung and G. Koretzky, unpublished data]. This is somewhat surprising since SLP-76 is inducibly phosphorylated in both macrophages and natural killer cells when receptors utilizing ITAMs are engaged. The lack of a functional defect in these populations suggests that other adapter proteins may serve the function of SLP-76 in these cells or that not all ITAM-bearing receptors share the same requirements for activating their downstream signaling machinery.

Interestingly, however, there is another significant abnormality in SLP-76-deficient mice. When heterozygous by heterozygous matings are established, instead of finding the expected 25% knockout mice, only 8% of SLP-76-deficient progeny survive into adulthood. This early mortality correlates with diffuse fetal hemorrhage in SLP-76-deficient mice [70, 73]. The bleeding diathesis continues into adulthood, as SLP-76 null mice demonstrate persistent peritoneal hemorrhage and mild anemia. Although platelet counts are slightly low (approximately 70% of normal), it is unlikely that thrombocytopenia is causal for the fetal and adult hemorrhage. Analysis of platelet function does reveal that platelets obtained from SLP-76-deficient mice fail to aggregate or release granule material in response to collagen, although platelet function as assessed by these parameters is normal following stimulation with thrombin. This correlates with the finding that

although collagen stimulation induces activation of the syk PTK, PLCγ2 phosphorylation is not seen in the SLP-76-deficient samples [73, 74]. These data suggest that in platelets, as in T cells, SLP-76 plays a critical role to couple syk family PTKs with downstream signaling events. Current investigations are underway to determine if this defect in platelet signal transduction is causal for the increased mortality of SLP-76-deficient mice.

Similar to SLP-76, LAT was initially identified as a Grb2-binding substrate of the TCR-induced PTKs [75]. Molecular cloning and subsequent analysis revealed that this relatively non-abundant protein is expressed in the detergent-insoluble glycolipid-enriched region of the plasma membrane (GEM) [76]. Like SLP-76, LAT is rapidly phosphorylated on tyrosine residues following engagement of the TCR. The LAT phosphotyrosine residues are in the correct orientation to bind the SH2 domains of several proteins known to be important for the propagation of T cell activation signals.

SLP-76 and LAT expression share a similar tissue distribution [65, 77]. Additionally, both molecules play critical roles in the regulation of T cell activation and development. Although overexpression of LAT in the Jurkat T cell line does not appear to augment TCR-mediated downstream activation events, studies of a LAT-deficient Jurkat mutant illustrate the critical role played by this molecule [78]. In this cell, TCR engagement fails to result in downstream activation events leading to interleukin-2 gene transcription. The biochemical signaling defect in the LAT-deficient cells is similar to that found in the SLP-76-deficient cell line described above in that the TCR fails to couple effectively to the phosphatidylinositol second messenger pathway. It was, therefore, not surprising when mice made deficient in LAT expression via homologous recombination were described to have a T cell phenotype identical to that of the SLP-76-deficient mice [79]. Similar to the SLP-76 knockouts, LAT −/− mice appear to have grossly normal macrophage and natural killer cell function. Interestingly, unlike SLP-76-deficient mice, mice lacking LAT do not demonstrate fetal hemorrhage or increased mortality.

In a more recent study, LAT was shown to form a multimolecular complex with the Fcγ receptor and the p85 subunit of phosphatidylinositol 3-kinase (PI3 kinase) following collagen treatment in platelets [80]. Additionally, it was shown that overexpression of LAT leads to increased antibody-dependent, cell-mediated cytotoxicity and natural cytotoxicity in natural killer cells [81]. Thus, both LAT and SLP-76 appear to play important roles in integrating signals mediated via a number of cell surface receptors on various hematopoietic cell types.

However, although SLP-76 and LAT play similar critical roles coupling engagement of the pre-TCR or mature TCR to downstream signaling events, it seems likely that these two molecules function quite differently at the molecular and biochemical level. Thus, there are important distinctions between these two molecules in terms of subcellular localization (LAT is a cell surface protein whereas SLP-76 is expressed in the cytosol) and in terms of the proteins with which each of these adapter molecules interact. While many of these intermolecular interactions have now been described, the importance of the various molecular complexes which are generated remains an area of intense investigation.

Two major tyrosine phosphorylation sites have been identified in the SLP-76 amino-terminal region. Interestingly, both of these tyrosines (Y113 and Y128) fall within an identical motif (DYESP) [82]. Several laboratories have shown that upon tyrosine phosphorylation of SLP-76, there is an inducible association with the SH2 domain of the protooncogene Vav [83–85]. This guanine nucleotide exchange factor for small-molecular-weight GTP-binding proteins plays a critical role in reorganization of the cytoskeleton following TCR engagement. Evidence has been presented suggesting that the trimolecular complex between SLP-76, Vav, and another adapter protein (Nck, which also binds to tyrosine-phosphorylated SLP-76) is critical for this cytoskeletal reorganization [86]. Although a series of experiments have demonstrated that SLP-76 and Vav act synergistically to augment TCR-mediated activation of the interleukin-2 gene, more recent evidence suggests that SLP-76 and Vav work in overlapping, but distinct pathways to reach this endpoint [87]. Thus, while it is possible that the association between SLP-76 and Vav is required for cytoskeletal changes following TCR engagement, it appears that the interaction between SLP-76 and Vav is not required for these two proteins to function synergistically to augment TCR-induced interleukin-2 production.

The central proline-rich region of SLP-76 was initially shown to bind to the Grb2 SH3 domains in in vitro assays. It was difficult, however, to demonstrate an association between SLP-76 and Grb2 in intact cells. An explanation was provided by several recent studies demonstrating that another Grb2 family member, Gads, otherwise known as GrpL, or Grf4O, appears to be the physiologically relevant binder of the proline-rich region of SLP-76 in T lymphocytes [80–90]. Interestingly, an identical cDNA was independently cloned by another group using the yeast two-hybrid screen to identify proteins that associate with autophosphorylation sites in the macrophage/monocyte colony stimulating factor (M-CSF) receptor (named MONA for monocyte adapter protein) [91]. Overexpression of MONA in bone marrow cells results in a reduction in

M-CSF-dependent macrophage production in culture, suggesting that MONA plays an important role in the regulation of macrophage/monocyte development. The observation that the Gads/SLP-76 complex can be detected in intact cells unlike the Grb2/SLP-76 complex is even more intriguing, as Gads has been shown additionally to inducibly associate with LAT. Collectively, these findings suggest the possibility that a SLP-76/Gads/LAT complex may play a role in integration of TCR-mediated signaling pathways.

The carboxy-terminal SH2 domain of SLP-76 associates inducibly with another substrate of the TCR-stimulated protein tyrosine kinases, SLP-76-associated phosphoprotein of 130 kDa (SLAP-130) [92], also known as fyn-binding protein, or Fyb [93]. SLAP-130, like SLP-76, is phosphorylated early upon TCR engagement. Unlike SLP-76, which appears to be a substrate of ZAP-70, SLAP-130 is phosphorylated by src family kinases. Controversy remains regarding the role of SLAP-130 as a regulator of T cell activation events. This 130-kDa molecule is an adapter protein with no known enzymatic activity. In addition to inducibly binding to SLP-76 via SLAP-130 phosphorylation sites and the SLP-76 SH2 domain, SLAP-130 associates constitutively with another family of adapter molecules, SKAP55 and its homologues [94–97]. SKAP-55 contains an amino-terminal PH domain which mediates its association with fyn while the carboxyl-terminal SH3 domain directs a constitutive association with SLAP-130. The functional significance of the multimeric complex of fyn/SLAP-130/SKAP-55 remains elusive but will likely prove to be an interesting modulator of signaling following TCR engagement. In some model systems, overexpression of SLAP-130 appears to interfere with SLP-76 function [91–98] while under other conditions, SLAP-130 appears to work synergistically with fyn along with SLP-76 to promote interleukin-2 gene transcription [99]. Further evaluation of these and other systems will be required to elucidate more precisely the role of SLAP-130 in the regulation of signal transduction and the relationship between SLAP-130 and SLP-76 as integrators of signaling cascades.

In addition to binding to Grb2 family members (including Gads), LAT associates with a number of other important signaling molecules in T cells following TCR engagement. These include PLCγ1 and PI3 kinase [75, 80]. It is clear, also, that LAT must be targeted to the plasma membrane, both via its transmembrane domain and fatty acid modifications for its most efficient function [76]. Additionally, LAT has been shown to localize within GEMs following TCR engagement. These data suggest that this adapter protein functions as a scaffold to bring together a number of important effector molecules within the TCR-initiated signaling complex.

Further structure/function analyses are required on both SLP-76 and LAT to determine the importance of the various intermolecular interactions described thus far. The development of SLP-76- and LAT-deficient cell lines as well as animals which lack either of these important adapter molecules will be critical reagents for these studies, which can now be performed in vitro, in intact cells, and in whole animals.

Other adapter proteins which act as positive regulators of lymphocyte activation

Tissue distribution studies reveal that, while expressed in T cells, natural killer cells, and macrophages, neither SLP-76 nor LAT is found in B lymphocytes. Concordant with this observation, B cell function is intact in SLP-76- and LAT-deficient mice [69, 70, 79]. Because there are so many similarities between antigen-receptor-mediated signaling events in B and T cells, it was likely that molecules similar to SLP-76 and LAT would be found to be important in B cell function. This has proven to be the case as demonstrated by the cloning and initial characterization of a SLP-76 homologue BLNK (also known as SLP-65 and BASH), a cytosolic adapter molecule which is phosphorylated rapidly upon engagement of the B cell antigen receptor [100, 101]. Similar to studies with SLP-76, overexpression of BLNK in B cell lines augments signal transduction events while the generation of a BLNK-deficient chicken B cell line demonstrates that BLNK is critical for the antigen receptor to couple with its downstream machinery [102]. Protein-protein interaction studies have demonstrated intermolecular interactions between BLNK and key effector molecules in B cell signal transduction pathways, suggesting that BLNK is a functional as well as structural homologue of SLP-76 [103, 104]. It is interesting, also, that BLNK is expressed in macrophages and may provide an explanation for normal signal transduction by SLP-76-deficient macrophages.

In addition to LAT, SLP-76, and related proteins, other adapter molecules also play important roles in lymphocyte activation. Among these are Shc, a ubiquitously expressed cytosolic protein which is phosphorylated upon engagement of antigen and cytokine receptors [105–107]. In a number of model systems, Shc plays an important role in coupling receptor PTKs with ras [61, 62]. In T cells, the importance of Shc in modulating this particular signaling pathway is less well defined. While Shc has been shown to inducibly associate with the ζ chain of the CD3 complex, recent studies suggest that this intermolecular interaction is not required for coupling the TCR with ras [108]. There is more compelling evidence to suggest that interleukin-2

receptor signaling utilizes Shc and Shc-associated proteins as it transduces its signals leading to cellular proliferation [107, 109–111].

Recently, a number of other adapter molecules, some of which are expressed exclusively in hematopoietic cells and others more ubiquitously, have been identified. These include Nck which, as noted above, may play a role in cytoskeletal reorganization along with SLP-76 and Vav. The Wiskott-Aldrich syndrome protein may play a key role in this process [112]. Further studies taking molecular, biochemical, and genetic approaches will be required to elucidate the role played by each of these molecules in the regulation of lymphocyte activation. Another recent study using the yeast two-hybrid screen to identify syk PTK-interacting proteins in B cells identified 3BP2 [113]. 3BP2 contains an amino-terminal PH domain, a central proline-rich motif, and a carboxyl-terminal SH2 domain. In addition to syk, the 3BP2 SH2 domain associates with ZAP-70, LAT, Grb2, PLCγ1, and Cbl in activated T cells. Overexpression of 3BP2 induces transcriptional activation of the interleukin-2 promoter and its NFAT and AP-1 elements [114]. Optimal activity of 3BP2 is dependent on both its SH2 and PH domains, and requires functional syk kinases, ras, and calcineurin. These studies suggest that 3BP2 may couple activated ZAP-70 or syk to a LAT-containing signaling complex important for TCR-mediated gene transcription.

Adapter proteins also function as negative regulators of lymphocyte activation

In addition to adapter proteins functioning as positive regulators of TCR-stimulated signaling pathways, evidence has been presented demonstrating that some adapters may also interfere with these second messenger cascades. One example involves Cbl [115], an adapter protein which contains an amino-terminal phosphotyrosine-binding domain, a proline-rich region, and several tyrosine residues which are inducibly phosphorylated following TCR engagement [116]. In contrast to SLP-76, which when overexpressed in T cells results in an augmentation of TCR-induced signaling events, transient overexpression of Cbl diminishes TCR-dependent AP-1 activation [117]. This is consistent with a previous observation that the Cbl homologue found in *Caenorhabditis elegans* (Sli-1) interferes with receptor-tyrosine-kinase-mediated activation of ras [118]. Figure 3 depicts two models for mechanisms by which Cbl may regulate TCR-mediated signaling events. The first is based on the observation that following TCR engagement, Cbl disassociates from Grb2 and binds instead to CrkL (another adapter protein) and C3G, a guanine nucleotide exchange factor for the small-molecular-weight GTP-binding protein, Rap-1 [119–123]. This results in activation of Rap-1 which binds to and sequesters Raf-1, the kinase immediately downstream of ras. This effectively prevents ras from binding and activating Raf-1, resulting in T cell unresponsiveness instead of activation following TCR engagement [124].

A second model for Cbl interference with activation events comes from the observation that in addition to binding the CrkL/C3G complex, Cbl also associates with other proteins in the cell. These include Vav, and the p85 subunit of PI3 kinase. Additionally, tyrosine-phosphorylated ZAP-70 and syk bind Cbl via the Cbl PTB domain [125–128]. The importance of this intermolecular interaction was revealed by studies demonstrating that when Cbl binds to syk family PTKs, the kinases are targeted for degradation, effectively terminating signal transduction events [129].

Recently, another transmembrane adapter protein (SHP-2-interacting transmembrane adapter protein, or SIT) was described. SIT is a disulfide-linked homodimer which associates with the TCR complex [130]. Sequence analysis revealed that instead of possessing an ITAM, SIT contains a cytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM). ITIMs are tyrosine-based motifs found in the cytoplasmic domains of many cell surface molecules that are also inducibly phosphorylated following receptor engagement. Instead of binding PTKs, however, ITIMs have been shown to recruit protein tyrosine phosphatases or lipid phosphatases, resulting in the termination of signaling events [131]. Following its tyrosine phosphorylation, SIT recruits the SH2-domain-containing tyrosine phosphatase, SHP-2. Since SHP-2 has been implicated as a negative regulator of lymphocyte activation, this observation suggested the possibility that SIT could interfere with TCR-mediated signaling. Support for this potential role came from overexpression studies where transfection of SIT into the Jurkat T cell line downregulates TCR as well as phytohemaglutinin-mediated activation of the NFAT response element of the interleukin-2 gene. Biochemical analysis suggests that the inhibition of TCR function occurs upstream of activation of PLCγl. Interestingly, however, it was shown that binding of SHP-2 to SIT is not required for the inhibition of NFAT activation, suggesting that the role of SIT in lymphocyte function is more complex than merely recruitment of a phosphatase to the TCR complex.

In addition to adapters serving negative regulatory roles by inducing protein-protein interactions, an interesting series of experiments have recently demon-

strated that adapters may interfere with signaling by blocking the recruitment of important effector molecules. Sap (for SLAM-associated protein) [132] was recently identified as a T-cell-specific protein possessing a single SH2 domain followed by a short carboxyl-terminal tail. The Sap SH2 domain allows Sap to be recruited to the plasma membrane following engagement and tyrosine phosphorylation of a transmembrane protein, Cdw-150, also known as SLAM. Binding of Sap to SLAM prevents the association of other SH2-domain-containing molecules with SLAM. Interestingly, in the absence of Sap, inducible phosphorylation of SLAM results in the recruitment of SHP-2, the tyrosine phosphatase described above. The biologic importance of Sap function has been underscored by the demonstration that mutations in the Sap gene, including those which interfere with the inducible association of Sap to SLAM, are causal for X-linked lymphoproliferative (XLP) syndrome [132, 133], a disorder characterized by uncontrolled expansion of B cell populations which have been infected with Epstein Barr virus [134]. It remains unclear whether the pathogenesis of this disorder relies upon the failure of Sap to compete with SLAM-associated SHP-2 or whether other protein-protein interactions are dysregulated. Further studies of the signal transduction defects in lymphocytes isolated from patients with XLP should shed light on the precise mechanism by which Sap functions in lymphocyte activation [135].

A more recent study investigated Sap function in natural killer cell activation [136]. Since SLAM is homologous with the mouse cell surface receptor 2B4, the human homologue of 2B4 was identified and shown to be tyrosine phosphorylated following pervanadate treatment of transfected cells leading to the recruitment of SHP-2. SAP was shown to be recruited to 2B4 in activated cells, and the 2B4-SAP interaction prevented the association between 2B4 and SHP-2. This study shows that in addition to SLAM, other cell surface molecules utilize SAP as a signaling adapter protein. This finding may also be important for understanding the pathogenesis of XLP. In this regard, another recent study provides evidence suggesting that engagement of SLAM also enhances lymphocyte apoptosis mediated via the CD95 signaling pathway [137]. This report showed an association between SLAM and SH2-domain-containing inositol phosphatase (SHIP), although the importance of the SLAM/SHIP interaction in the regulation of signaling events remains unknown.

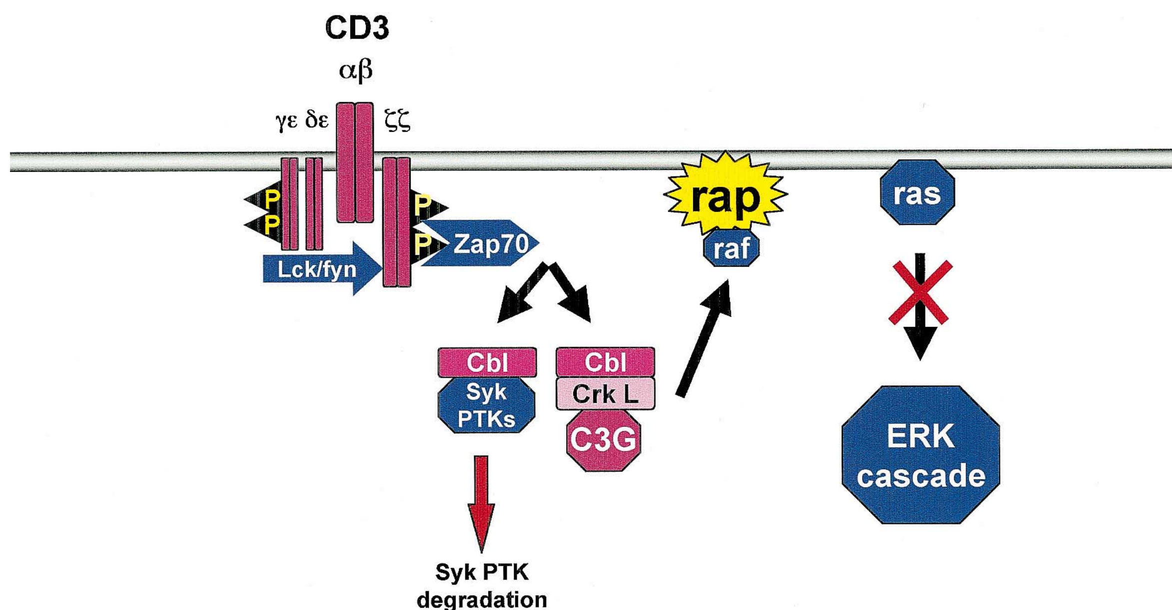

Figure 3. Schematic illustration of signals thought to inhibit TCR-induced signaling. Following TCR ligation, ZAP-70 phosphorylates Cbl promoting the association of Cbl with target proteins such as CrkL and syk family PTKs. Association of Cbl with the syk family PTKs leads to the degradation of the kinases, thereby blocking further signal transduction. Formation of the Cbl/CrkL/C3G complex promotes the activation of Rap-1.

denotes phosphorylated tyrosine residues.

Summary

In recent years, many components of the signal transduction machinery linking antigen recognition with downstream biologic events have been identified and characterized. These include the antigen receptors, the enzymes responsible for initiating signaling events and, most recently, adapter molecules which mediate important protein-protein interactions. While additional effector and adapter molecules certainly have yet to be identified, one of the major challenges facing investigators interested in lymphocyte signal transduction is to examine the cell biology of the molecular scaffolds upon which signaling complexes are built. This will involve investigations into the spatial localization and temporal formation of multimeric molecular complexes with precise attention to which molecules are included and which precluded from the various signaling complexes. These studies will continue to make use of the genetic, molecular, and biochemical tools currently being employed to identify the components of the signal transduction cascades, as well as sophisticated imaging techniques to visualize the molecular complexes in real time in intact cells.

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