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Signaling mechanisms utilized by antigen receptors and integrins: common intermediates, different outcomes?

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1. ABSTRACT

Antigen receptors and integrins are structurally and functionally distinct, but both play key roles in regulating immune cell activation and function. Understanding the molecular basis of the signaling pathways utilized by antigen receptors and integrins is fundamental to identifying the mechanisms underlying immune system function and dysfunction (e.g. autoimmune disease) and identifying potential targets for modifying the immune response with therapy. Recently, several key regulators of antigen receptor signaling have also been revealed to be important molecular intermediates in integrin-triggered signaling pathways. These include the protein tyrosine kinase Syk, the guanine nucleotide exchange factor Vav, and the adaptor protein SLP-76. While antigen-receptor signaling is generally associated with leukocyte activation and differentiation, integrins are most commonly thought of as adhesive receptors. This raises the interesting question of how common molecular intermediates may regulate diverse cellular processes such as activation versus adhesion and migration, and provides a framework for defining potentially unique mechanisms utilized by cells of the immune system to regulate integrin-dependent cell function.

2. INTRODUCTION

Naïve T cells continually sample a spectrum of peptides derived from both self-tissues and foreign pathogens presented in the context of major histocompatibility complexes (MHC) expressed on the surface of antigen presenting cells (APC). While MHC-peptide (MHC:p) complexes derived from self are ignored or promote immune tolerance, MHC molecules bearing peptides generated from foreign sources elicit potent activation of T cells expressing an antigen-specific T cell receptor (TCR) (1). Productive engagement of the TCR leads to a cascade of signaling events that ultimately dictate the capacity of T lymphocytes to respond to a given pathogen and direct a productive immune response (2). During the past ten years, adaptor proteins have gained increasing recognition as critical molecular intermediates in multiple signaling pathways triggered upon antigen receptor ligation. More recently, adaptor proteins have again emerged as key regulators governing integrin signaling in cells of the immune system. As the name applies, adaptor proteins function as molecular scaffolds for the assembly and localization of macromolecular signaling complexes within the cell. The capacity to engage multiple signaling partners contributes to the ability of adaptor

proteins to participate in many signaling pathways, including those regulating the cell cytoskeleton. Here, we review the relatively well-defined role of the adaptor proteins SLP-76 (SH2-domain containing leukocyte protein of 76 kD), Gads, and LAT (linker for activation of T cells) in propagating antigen-receptor signaling, and use this as a background for discussing the more recently appreciated role for SLP-76 and SLP-76-associated proteins in regulating integrin signaling in hematopoietic cells.

3. FORMATION OF THE IMMUNOLOGICAL SYNAPSE REQUIRES A COLLABORATIVE EFFORT BETWEEN THE TCR, INTEGRINS AND COSTIMULATORY MOLECULES

Antigen-specific activation of T lymphocytes is a highly regulated process that involves the coordinated recruitment of multiple surface receptors and intracellular signaling molecules to the contact site between the T cell and the APC. Initial contacts between a T cell and APC are transient and relatively independent of the TCR, and are most likely mediated by accessory molecules such as CD2, a member of the ICAM family of adhesion molecules, and by integrins like LFA-1 (reviewed in (3). Weak adhesions of this type allow the T cell to scan the surface of the APC in search of agonist MHC:p complexes. Should the T cell find no cognate MHC:p complexes expressed on the surface of the APC, the T cell will break off and continue to interrogate neighboring APCs or leave the lymph node. Specific recognition of MHC:p complexes present on the surface of an APC by the TCR, coupled with the appropriate costimulatory signals leads to an increase in actin polymerization and the formation of a specialized signaling structure called the immunological synapse (IS) (4). Maturation of the IS is characterized by the segregation of membrane molecules into two distinct structures, the central supramolecular activation cluster (cSMAC) where surface receptors and signaling molecules such as TCR, CD28, CD2, Lck and PKC-theta are localized, and the peripheral supramolecular activation cluster (pSMAC) containing the integrin LFA-1 and the actin binding protein talin (5,6). It is believed that compartmentalization of receptors and intracellular signaling molecules provides a platform for localized, productive signaling through the TCR. This is supported by the observation that failure to form an IS results in suboptimal or abortive T cell activation (7).

While the relative contributions of integrin-dependent signaling and TCR-triggered signaling to the early phases of IS formation are not entirely clear, two recent studies have elegantly demonstrated that TCR signaling occurs prior to the cytoskeletal rearrangements that actually lead to formation of the IS (8,9). This indicates that a productive signal triggered by cognate interactions between the TCR and MHC:p does play an active role in regulating the cytoskeletal changes that drive formation of the IS. Changes in the conformation and localization of integrins such as LFA-1 in response to TCR stimulation is a form of "inside-out" signaling that increases the affinity and avidity of LFA-1 for its ligands ICAM-1/2 on the APC and requires an intact cytoskeleton and the actin-binding protein talin (10-14). Further signaling generated by adhesion receptors and co-stimulatory molecules such as CD2 and CD28 induces additional actin polymerization and cytoskeletal rearrangements. In fact, several studies have shown that costimulation via CD28 is required for sustained actin polymerization and SMAC formation (15,16).

4. MULTIPLE ADAPTOR PROTEINS ARE SUBSTRATES OF PTKS ACTIVATED BY ANTIGEN RECEPTOR LIGATION

It has been appreciated for some time that the activation of protein tyrosine kinases (PTKs) occurs rapidly following antigen receptor ligation, and that PTK activation is required for subsequent signaling. Identification of specific PTK substrates phosphorylated following antigen receptor engagement became critical to understanding how the proximal activation of PTKs was coupled with more distal signaling events, such as gene activation. To this end, several groups described the inducible phosphorylation of tyrosine residues in specific motifs located in the intracellular portions of multiple antigen-receptor associated polypeptides, including the TCR-associated zeta-chains and CD3 complex (gamma, delta and epsilon chains) (17,18). Each of these ~ 26 amino acid motifs contains two tyrosine residues flanked by specific amino acids (YxxK---YxxK), and are most commonly referred to as immunoreceptor tyrosine based activation motifs (ITAMs). ITAMs are also present in the cytoplasmic tails of multiple signal-transducing antigen receptor subunits, including the B cell receptor (BCR)-associated Ig-alpha and Ig-beta chains and the Fc receptor-associated beta and/or gamma and zeta chains. ITAMs are phosphorylated by Src-family PTKs (e.g. Lck), and serve as membrane-associated docking sites for proteins with SH2 domains, including ZAP-70 and Syk PTKs. Both Syk and ZAP-70 have two SH2 domains, which bind in tandem to the phosphorylated tyrosines in a single ITAM. Upon binding to ITAMs, Syk family PTKs are activated, phosphorylate numerous substrates and further propagate the signaling cascade. Multiple inhibitory receptors (e.g. inhibitory Fc receptors, CTLA-4) contain analogous intracellular motifs called ITIMs (immunoreceptor tyrosine-based inhibitory motifs), which are also substrates of Src PTKs. Phosphorylation of ITIMs results in the recruitment of tyrosine or inositol phosphatases (e.g. SHP-1, SHIP), inhibition of intracellular calcium flux and a concomitant inhibition of downstream signaling (19,20).

While the requirement for inducible PTK activity for transducing intracellular signals following TCR ligation became generally accepted, the precise mechanisms by which PTK activation was translated into more distal signaling events remained elusive for some time. Several laboratories therefore undertook the task of identifying specific substrates of antigen-receptor activated PTKs that might function to propagate more distal signaling events following antigen-receptor ligation. Consequently, SLP-76 was identified and partially characterized based on affinity for a GST-Grb2 fusion protein. LAT was also originally identified as one of the predominant substrates of one or

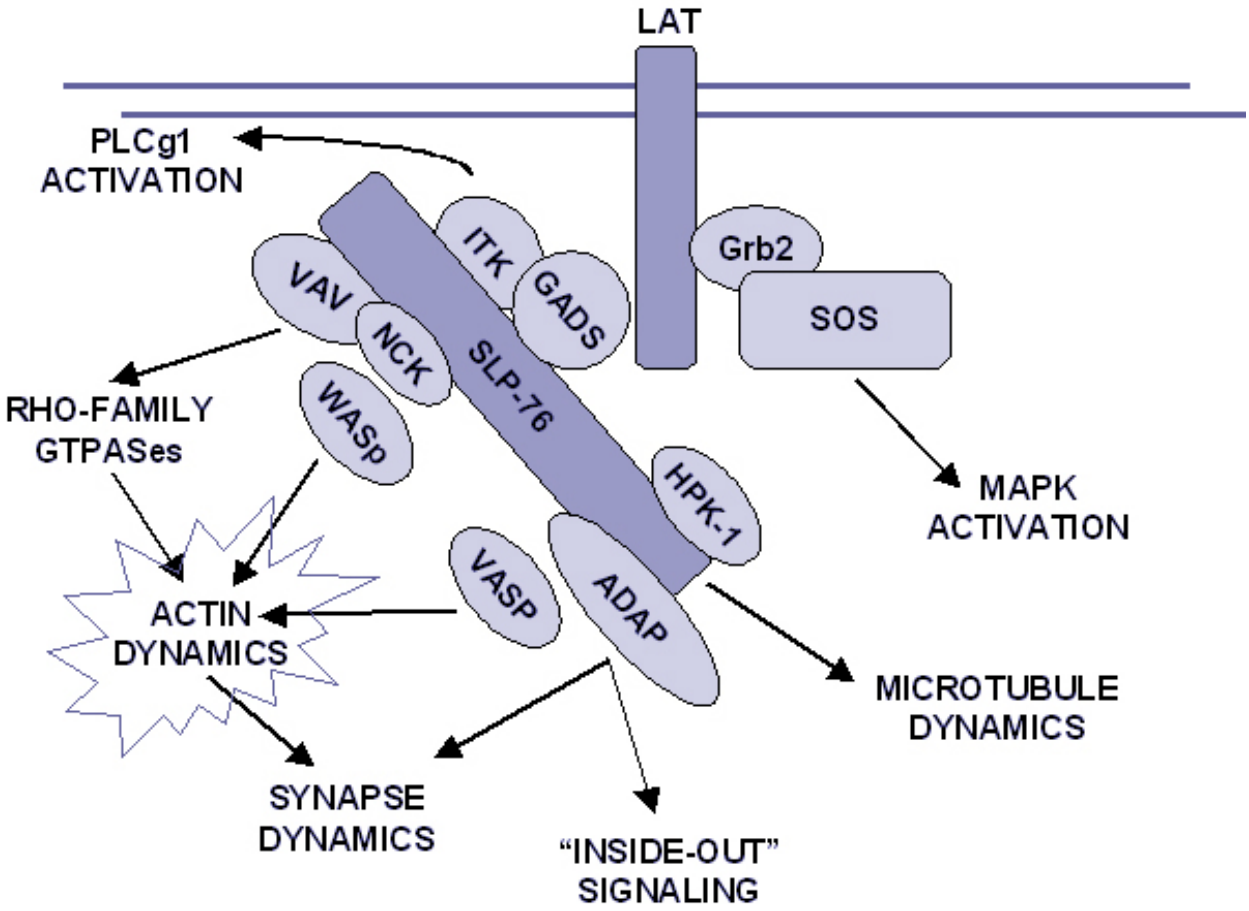

Figure 1. The adaptor proteins SLP-76 and LAT couple TCR ligation with multiple downstream signaling events. In resting T cells, SLP-76 associates with the adaptor protein Gads in a constitutive manner. Following TCR ligation and PTK activation, both SLP-76 and LAT are phosphorylated on tyrosines, resulting in the recruitment of multiple signaling intermediates. For SLP-76, these include Nck, Vav, Itk, and PLCg1. In addition, HPK-1 and ADAP are also tyrosine phosphorylated, and bind to the SH2-domain of SLP-76. Once phosphorylated, LAT recruits Gads and the SLP-76 complex to the inner leaflet of the plasma membrane. LAT also recruits PLCg1 and a Grb2/Sos complex. The more distal signaling events regulated by SLP-76 and/or LAT-nucleated signaling complexes are depicted.

more PTKs activated following TCR ligation. One of the most striking features of both SLP-76 and LAT is the complete absence of any enzymatic domains or activity. SLP-76 consists of multiple domains that associate either constitutively or inducibly with additional signaling proteins in T cells. These domains include an acidic N-terminal domain that contains three tyrosine residues phosphorylated upon TCR ligation (in human SLP-76, $Y^{113, 128, \text{ and } 145}$), a central proline rich domain, and a C-terminal SH2 domain (21). LAT is targeted to lipid rafts within the plasma membrane by virtue of two cysteine residues that are palmitoylated post-translationally (22). The extracellular domain of LAT is very small, and no known ligand for LAT exists. The cytoplasmic domain of LAT contains multiple tyrosine phosphorylation sites, including four key tyrosine residues that have been implicated in multiple aspects of T cell development, activation, and differentiation (23-29). The fact that both SLP-76 and LAT function solely as adaptors became even more remarkable as a greater appreciation of the critical role for these adaptors in propagating multiple aspects of TCR-dependent signaling was realized.

4.1. The adaptor proteins LAT, Gads, and SLP-76 are critical signaling intermediates in multiple pathways following TCR ligation

Since their initial identification and description in 1994, SLP-76 and LAT have proven to be very informative focal points for defining the signaling pathways coupling TCR ligation with more distal signaling events in T cells. Based on elegant studies conducted by multiple laboratories, a more complete picture of how these adaptors link TCR-activated PTKs with more distal signaling events, including actin polymerization and cytoskeletal dynamics, has emerged (Figure 1). Upon TCR stimulation, Src family PTKs such as Lck and Fyn are rapidly activated and phosphorylate ITAMs of the TCR-associated zeta-chains. The Syk family PTK ZAP-70 binds the phosphorylated zeta-chains via its SH2 domain and is activated in trans by Src-family PTK mediated phosphorylation and by

autophosphorylation (30,31). Both SLP-76 and LAT are immediate substrates of activated ZAP-70, and once phosphorylated, serve as docking sites for multiple adaptor proteins and signaling molecules. For SLP-76, these include the adaptor proteins Nck, Gads, and SLAP-130/FYB. Given demonstrated roles in regulating leukocyte adhesion and granule release (32,33), SLAP-130/FYB has more recently been designated the Adhesion and Degranulating promoting Adaptor Protein (ADAP), and we will use this designation for the remainder of this article. SLP-76 also associates with the guanine nucleotide exchange factor (GEF) Vav, the PTK Itk, PLCg1, and the serine/threonine kinase HPK-1. Phosphorylated LAT recruits PLCg1, the p85 subunit of PI3-kinase, and a pre-formed complex containing the adaptor protein Grb2 and the GEF Sos. SLP-76 and LAT are therefore central players in multiple signaling pathways governing second messenger generation, MAP kinase activation, and gene transcription following TCR ligation. Indeed, mutant lines of Jurkat T cells lacking SLP-76 or LAT manifest severe and overlapping defects in multiple TCR-mediated signaling events, including impaired or reduced calcium flux, NF-AT transcriptional activity, and phosphorylation of PLCg1 and p44/42 MAP kinases (ERK) (24,28,34). The observation that SLP-76 deficient Jurkat cells are defective in ERK phosphorylation and AP-1-mediated gene transcription in the context of normal LAT phosphorylation suggests that SLP-76 and LAT function in concert to regulate optimal MAP kinase (MAPK) phosphorylation following TCR ligation.

4.2. SLP-76 is recruited to LAT and the cell membrane via the adaptor protein Gads

While SLP-76 was identified originally based on affinity for the SH3 domains of Grb2, SLP-76 associates *in vivo* with the Grb2-related adaptor Gads in a constitutive manner (35). Like Grb2, Gads contains a single SH2 domain flanked by SH3 domains. Gads was initially identified using phosphopeptides derived from Shc to screen an expression library, and the *in vivo* interaction between Gads and SLP-76 was revealed soon after (36). Following TCR ligation, the SH2 domain of Gads binds to a phosphorylated tyrosine in LAT, resulting in recruitment of the Gads/SLP-76 protein complex to membrane-associated LAT (35,37). This is a critical event for TCR signaling, as a SLP-76 mutant that cannot associate with Gads fails to rescue many aspects of TCR signaling in a SLP-76 deficient Jurkat T cell line. This same mutant, when expressed as a chimeric protein linked to the extracellular and transmembrane domains of LAT, is sufficient to restore TCR signaling in a LAT-deficient Jurkat T cell line (JCam.2), supporting the idea that LAT, Gads, and SLP-76 are major components of a biochemical circuit connecting TCR ligation with more distal signaling events (38,39).

A more detailed understanding of the precise contributions of the Gads/SLP-76 interaction to TCR signaling was realized with the definition of the specific amino acids of SLP-76 which mediate Gads binding. Using peptide arrays, the minimum amino acid sequence (RxxK) required for Gads recruitment was identified (40). This sequence resides within the proline rich domain of SLP-76, but is distinct from the region of SLP-76 (P1 domain) that interacts with the SH3 domain of PLCg1 (41). Mutation of either the P1 or Gads-binding domain inhibits the capacity of ectopically expressed SLP-76 to correct the defects in TCR signaling in a SLP-76 deficient Jurkat T cell line (42). Furthermore, expression of the Gads-binding domain of SLP-76 disrupts the endogenous association of SLP-76 and Gads, and impairs TCR-triggered re-localization of SLP-76 within the cell. Specifically interfering with the Gads/SLP-76 interaction in the context of intact PLCg1 binding results in impaired calcium flux and CD69 upregulation, indicating that Gads-mediated re-localization of SLP-76 is critical for SLP-76 function. Presently, it is not clear which adaptor (LAT or SLP-76) is primarily responsible for recruitment of PLCg1 to the plasma membrane following TCR ligation. It seems likely that SLP-76 and LAT function in concert to promote the optimal phosphorylation and membrane localization of PLCg1 following TCR ligation, as SLP-76 associates with the PTK Itk (a known activator of PLCg1), and both LAT and SLP-76 can bind to PLCg1. Interestingly, replacing the endogenous allele of LAT with a copy encoding a point mutant of LAT (Y^{136}) that fails to bind PLCg1 results in lymphoproliferation and skewing of the CD4+ T cell subset toward a TH2 phenotype, suggesting that direct recruitment of PLCg1 to LAT may be important for generating signals regulating programmed cell death and T cell homeostasis (23,26).

Mutant strains of mice lacking SLP-76, Gads or LAT have also been described. Both the SLP-76 and LAT deficient strains manifest a remarkably similar and severe block in thymocyte development, and are therefore virtually devoid of mature thymocytes and peripheral T cells (29,43,44). Curiously, Gads deficient mice also demonstrate defects in thymocyte development, but not to the same extent as that observed in the absence of LAT or SLP-76 (45). This suggests that 1) Gads is not strictly required for coupling SLP-76 with pre-TCR signaling in developing thymocytes, or 2) the existence of a compensatory molecule (perhaps Grb2?) that can at least partially substitute for Gads during pre-TCR dependent selection. The former possibility is supported by the observation that a SLP-76 mutant incapable of binding Gads partially restores thymocyte development in SLP-76 -/- mice (46,47). Surprisingly, the SH2 domain of SLP-76 is largely dispensable for pre-TCR mediated thymocyte development, as the CD4/CD8 profile and thymocyte number is comparable between SLP-76 -/- mice reconstituted with wild type SLP-76 or SH2-domain mutants of SLP-76 (46,47). This observation is consistent with the fact that T cell development is largely unaffected in mice lacking ADAP, which binds the SH2 domain of SLP-76 (33,48). Given the profound effects of mutating the SLP-76 binding domain in ADAP on the ability of ADAP to promote T cell:APC conjugate formation and LFA-1 clustering (49) , it is tempting to speculate that early thymic differentiation driven by the pre-TCR is not dependent on TCR-mediated "inside-out" signals that modulate integrin avidity. In support of this idea, pre-TCR mediated thymic development does not require the

extracellular immunoglobulin-like domains of the pre-TCR, suggesting that the assembly of a pre-TCR complex may be sufficient to promote intracellular signals required for differentiation (50). Peripheral T cells isolated from SLP-76 -/- mice reconstituted with either the Gads-binding domain or SH2 domain mutants manifest severe defects in TCR-induced proliferation and PLCg1 phosphorylation, indicating a more stringent requirement for these domains in the activation of mature T cells following TCR ligation (46,47). In contrast to the Gads-binding domain and SH2 domain mutants, thymocyte development remains substantially impaired in SLP-76 deficient mice expressing SLP-76 mutants where all three phosphorylated tyrosine residues are replaced (Y3F) or lacking the entire N-terminal acidic domain, suggesting that the molecular interactions taking place within the acidic domain of SLP-76 are crucial for driving pre-TCR dependent development (46,47).

4.3. SLP-76: a molecular link bridging TCR ligation with the actin cytoskeleton

By bridging the adaptor protein Nck and the GEF Vav, SLP-76 couples TCR ligation and PTK activation with several potent modulators of the actin cytoskeleton. Indeed, a direct effect of SLP-76, Vav, and Nck on actin cytoskeleton dynamics was suggested by the observation that overexpression of each of these proteins in Jurkat T cells augmented actin polymerization and p21-activated kinase 1 (PAK1) activation following TCR ligation (51). PAKs are known regulators of cytoskeletal dynamics and may be activated by GTP-bound Rac and Cdc42, products of Vav GEF activity (52,53). The enhancement of PAK1 activity seen in T cells overexpressing SLP-76, Vav and Nck was lost when mutations that prevented assembly of the SLP-76/Vav/Nck complex were introduced into the transfected constructs, suggesting that SLP-76 is essential for PAK1 activation. However, it should be noted at this point that PAK1 localization and activation is not solely dependent on SLP-76 in T cells following TCR ligation, as two studies have demonstrated PAK activation by the GEF PIX independent of SLP-76 and Vav (54,55).

More recently, SLP-76 has been shown to be required for recruitment of Nck and WASp (Wiskott Aldrich Syndrome protein) to the TCR following plating of Jurkat T cells on coverslips pre-coated with antibodies to CD3 (56,57). Upon activation by Cdc42 or Rac, WASp associates with the Arp2/3 complex and initiates de novo actin polymerization (57). The current model suggests that while LAT, ZAP-70, SLP-76, Nck, and WASp are all recruited to sites of initial TCR signaling, only SLP-76, Nck, and WASp remain colocalized at later time points and segregate from the TCR as Jurkat cells spread on stimulatory coverslips. Thus, SLP-76 and LAT serve similar roles in regulating the recruitment of signaling intermediates to the TCR upon initial contact, but may diverge in their functions as they relate to more distal signaling events at later time points. Additionally, WASp recruitment to the IS is at least partially dependent on CD2 and CD2 associated proteins such as CD2AP and PSTPIP1, indicating that more than one mechanism of WASp membrane localization exists during IS formation (58). WASp deficient T cells exhibit reduced actin polymerization and fail to form TCR caps when stimulated with CD3 antibodies (59,60). Interestingly, the ability of WASp deficient T cells to cluster LFA-1 following TCR stimulation is intact, providing evidence that clustering of the TCR and integrins are regulated by distinct pathways (61). Vav deficient T cells exhibit a strikingly similar phenotype to WASp deficient T cells with respect to actin polymerization induced by the TCR. Like WASp deficient T cells, Vav deficient T cells fail to cluster TCRs and actin cap formation is defective following TCR stimulation, suggesting overlapping functions of WASp and Vav (62-65). However, unlike WASp deficient T cells, integrin clustering is also defective in the absence of Vav1, indicating that Vav1 is also involved in this process (61). The localization of Vav to the TCR following receptor stimulation is an important aspect affecting Vav function and studies suggest that SLP-76 may play a role in the TCR-induced membrane recruitment of at least a pool of Vav. Using a SLP-76 deficient T cell line, several groups have shown that SLP-76 is actually required for membrane localization and activation of both Vav1 and Vav3 following TCR ligation (66,67). Additionally, in Jurkat T cells, expression of a mutant form of SLP-76 that is unable to bind Vav resulted in decreased Vav recruitment to the T cell/APC contact site as well as a decrease in the local concentration of activated Cdc42 at this site (68).

While these pioneering studies have revealed a dynamic re-localization of multiple signaling intermediates to the contact site of clustered TCRs and a stimulatory surface, the precise molecular ordering and contribution of SLP-76 and LAT to the initial and later stages of IS formation remain to be elucidated. SLP-76 may contribute to TCR-triggered formation of the IS and the SMAC via regulation of the intracellular localization of the adaptor protein ADAP. ADAP is also phosphorylated on tyrosines following TCR ligation (by Src-family PTKs), and binds to the SH2 domain of SLP-76 (69,70). By binding to SLP-76, ADAP may bring VASP and the VASP-associated actin monomer-binding protein profilin into a complex with Nck and WASp (71,72). Profilin may provide WASp and Arp2/3 with the actin monomers necessary for actin nucleation, but this has yet to be formally demonstrated. T cells isolated from ADAP-deficient mice manifest a defect in TCR-dependent "inside-out" signaling, resulting in impaired clustering of LFA-1 and suboptimal T cell activation following TCR ligation (33,48). Supporting it's role in modulating integrin avidity, enhanced ADAP expression in a T cell hybridoma augments the formation of T cell:APC conjugates and LFA-1 clustering, both of which are dependent on an intact SLP-76 binding site in ADAP (49). Furthermore, expression of the SLP-76-binding mutant of ADAP disrupts LFA-1 localization into the pSMAC (49). Collectively, these data suggest that SLP-76 and ADAP function in concert to regulate TCR-dependent early changes in LFA-1 avidity that may be required for subsequent maturation of the IS and the formation of the pSMAC.

The Tec family kinase Itk, an additional molecule that binds SLP-76 in an inducible manner following TCR engagement, also has the potential to influence T cell actin

cytoskeletal dynamics (73-75). Traditionally, it was thought that the main function of Itk was to promote TCR-induced phosphorylation and activation of PLCg leading to a rise in intracellular Ca^{2+}. In more recent years, examination of Itk deficient mice has revealed a function for Itk in TCR-induced actin polymerization and polarization. Surprisingly, the kinase activity of Itk was shown to be dispensable for its effects on the actin cytoskeleton. Instead, an intact SH2 domain appears to be all that is required for its function in this capacity (74,76). Interestingly, the Itk SH2 domain was shown to be necessary for the efficient recruitment of Vav to the TCR following receptor stimulation. As both Vav and Itk bind SLP-76, it is possible that Itk stabilizes the association between SLP-76 and Vav, thereby enhancing the membrane localization of this complex perhaps by binding membrane associated phosphatidylinositol (3,4,5)-trisphosphate via its pleckstrin homology (PH) domain (77). In addition, the activation of WASp and Cdc42 at the IS is reduced in the absence of Itk, most likely due to a lack of Vav recruitment (74). SLP-76, PLCg1, and Itk have also been shown to form a complex with LAT, and LAT is required for optimal activation of Itk and PLCg1 as well (78,79). Thus, as for PLCg1, SLP-76 and LAT appear to cooperate in the localization and activation of Itk, which may in turn have a direct impact on both PLCg1 activation and cytoskeletal dynamics. Indeed, studies using a LAT-deficient T cell line show a severe reduction in sustained spreading and actin polymerization elicited by the TCR (80).

4.4. Signaling via the BCR and Fc receptors: similar mechanisms, different adaptors

B cells appear to have evolved adaptor proteins that are distinct to but similar in structure and function to SLP-76 and LAT. Signals generated by the BCR are propagated by the SLP-76 homologue SLP-65/BLNK (B-cell linker protein). SLP-65 is expressed in B cells and macrophages and functions similarly to SLP-76 by associating with Vav1 and Nck, and regulating the activation of PLCg and the Tec family kinase Btk following BCR ligation (81-83). Like SLP-76 in T cells, the expression of SLP-65 in B cells is required for BCR-induced Ca^{2+} flux, PLCg phosphorylation, and ERK activation (84). Unlike SLP-76, SLP-65 is recruited to lipid rafts following BCR stimulation in a LAT independent manner, perhaps via direct interactions with the Ig-alpha-chain. However, the data are conflicting regarding the requirement of the Ig-alpha-chain ITAM in mediating this association (85,86). A more recent report demonstrates that a portion of SLP-65 is constitutively associated with the plasma membrane in an Ig-alpha-independent manner via a highly conserved leucine zipper located in the SLP-65 N-terminus (87).

The mechanisms by which hematopoietic adaptor proteins such as SLP-76 promote Fc receptor signaling are not as well defined and appear to be cell type-specific. In mast cells, SLP-76 and LAT have been shown to be required for efficient degranulation, Ca^{2+} flux, PLCg phosphorylation, and cytokine production following stimulation via the Fc-epsilon-RI receptor (88,89). However, in NK cells antibody-dependent cellular cytotoxicity was shown to be independent of SLP-76 (90). In neutrophils, SLP-76 appears to be involved in Fc-gamma-receptor signaling, as SLP-76 is phosphorylated and redistributes from the cytosol to the plasma membrane after Fc-gamma-receptor cross-linking. Additionally, SLP-76 deficient neutrophils exhibit impaired Ca^{2+} flux and reactive oxygen intermediate production upon immune complex stimulation (91). Conversely, Fc-gamma-receptor signaling in macrophages was shown to be independent of SLP-76 and its homologue SLP-65 altogether (92). These discrepancies may be due to the differential expression of Fc-gamma-activating and inhibitory receptors by the different cell types, or possibly indicates the existence of a yet unidentified SLP-76 homologue.

5. INTEGRIN SIGNALING: AN OVERVIEW

5.1. Focal adhesions and integrin signaling in non-hematopoietic cells

As discussed previously, the contribution of specific integrins (e.g. LFA-1) to the formation and maturation of the immunological synapse is widely accepted. However, to this point, the involvement of integrins has been discussed only in the context of TCR-dependent "inside-out" signals that regulate integrin avidity and adhesive function. What about "outside-in" signals generated by integrin ligation, which are also critical for multiple aspects of immune cell function? Like antigen receptors, integrin signaling is remarkably complex (93). Two of the most well defined events following integrin ligation are activation of Src-family PTKs and phosphorylation of the focal adhesion kinase (FAK). The FAK family of kinases consists of FAK, which is ubiquitously expressed, and Pyk2, which exhibits a more restricted pattern of expression that includes brain and and cells of hematopoietic origin (94). Phosphorylated FAK was initially identified in focal adhesions generated by fibroblasts and its phosphorylation was soon linked directly to the clustering of integrin receptors (95-97). Since that time, numerous studies have detailed a sizable list of FAK associated proteins and have also implicated FAK in the regulation of many signaling cascades activated by integrin ligation. FAK consists of an N-terminal FERM (protein 4.1, ezrin, radixin and moesin homology) domain, a kinase domain, proline rich regions and a FAT (focal adhesion targeting) domain at its C-terminus (98). Based on studies conducted in non-hematopoietic cells types, a mechanism by which FAK propagates signals from integrin receptors has been proposed. Following integrin ligation, the autophosphorylation activity of FAK increases, resulting in phosphorylation on Tyr^{397} (99). This promotes the SH2 domain mediated recruitment of Src and Src-family PTKs (100,101). Src further phosphorylates FAK at additional tyrosines such as Tyr^{576}, Tyr^{577}, Tyr^{861} and Tyr^{925} and fully activates FAK kinase activity (reviewed in (102). These phosphorylated residues provide docking sites for additional signaling molecules such as PLCg, the p85 subunit of PI3-Kinase and the p120RasGAP (GTPase activating protein) (102). FAK localization to focal contacts has been attributed to the binding of the FAT domain to other proteins such as talin and paxillin

(103,104). These proteins bind the FAT domain as well as to integrin receptors providing a possible mechanism of FAK membrane recruitment (105-107). The FAT domain also contains a binding site for the adaptor Grb2, providing one potential link between FAK and activation of the MAPK pathway. More recently, FAK was also shown to promote Ras activity by inducibly binding p120RasGAP in a phosphotyrosine dependent manner (108). The C-terminal region of FAK contains two proline-rich regions that support the binding of SH3 domain-containing proteins such as p130Cas and p190RhoGEF (109,110). P190RhoGEF and p130Cas may therefore contribute to FAK-mediated regulation of the actin cytoskeleton by promoting the activation of Rho and Rac, respectively (111,112).

Interestingly, studies of FAK deficient cells have determined that FAK is required for efficient focal adhesion turnover but not for the initiation of adhesion. FAK deficient fibroblasts exhibit enhanced adhesion and reduced motility, perhaps due to the formation of abnormally large and stable focal adhesions (113). In addition to being large, the focal adhesions formed in FAK-null fibroblasts remain "immature" and do not connect to actin stress fibers. FAK-mediated focal adhesion turnover may be regulated at the level of Rho activity as FAK associates with multiple proteins implicated in focal adhesion turnover, including the GTPase activating proteins GRAF and ASAP1, which may decrease Rho GTPase activity at focal adhesions (114,115). FAK may also induce focal adhesion turnover through the stabilization of microtubules, perhaps via Rho-dependent activation of mDia (mammalian diphanous), a protein that influences microtubule stabilization in a positive fashion (116). More recently, FAK has been implicated in the turnover of focal adhesions independent of the activity of Rho, but in conjunction with microtubules. In a study of fibroblasts, the formation of a complex containing FAK, the large GTPase dynamin and the adaptor protein Grb2 was shown to be necessary for microtubule-induced focal adhesion disassembly. Inhibition of dynamin caused reduced motility and the production of large focal adhesions, mimicking FAK deficiency (117). FAK may also mediate focal adhesion turnover at the level of calpain activation via the ERK pathway. Calpain is a calcium dependent protease that is activated following integrin stimulation and whose activity is also modulated by ERK (reviewed in (118). Substrates of calpain include, among others, actin-binding proteins such as alpha-actinin, FAK and integrin beta-tails. Calpain may participate in the dissolution of adhesion structures by severing the link between adhesion receptors and the cytoskeleton and by inducing the disassembly of signaling complexes (119-121). FAK binds to MEKK1 in focal adhesions and fibroblasts deficient for either FAK or MEKK1 have decreased calpain activation and defective rear-end detachment during migration (122). These data coupled with the observation that integrin-induced FAK phosphorylation in fibroblasts and platelets requires an initial round of actin polymerization suggests that a FAK-independent pathway couples integrin ligation to the very early phases of actin polymerization (123,124).

5.2. FAK and Pyk2-mediated integrin signaling in hematopoietic cells

In hematopoietic cells, the roles of the FAK family members in integrin signaling are just beginning to be defined. The requirement for FAK and/or Pyk2 in the propagation of integrin signals in hematopoietic cells appears to be somewhat cell-type and receptor-specific. In T cells, VLA-4 (alpha4beta1) activation of LFA-1-mediated migration is diminished by blockade of FAK or Pyk2 activity (125). In Pyk2 deficient mice, there is severe lack of marginal zone B cells, attributed most likely to poor B cell chemotaxis (126). Pyk2 was found to couple beta2 integrin ligation with activation of the ERK cascade and subsequent natural cytotoxicity in NK cells (127). NK cell transendothelial migration as well as migration through ICAM-1 and VCAM-1 coated filters was also shown to regulated by Pyk2 kinase activity presumably via Pyk2-mediated integration of chemokine and integrin receptor signals (128). Pyk2 also appears to control the chemotaxis of neutrophils to IL-8 as overexpression of a kinase dead mutant of Pyk2 inhibits this process (129). Interestingly, despite the fact that Pyk2 has the ability to mediate Rho GTPase activity on its own, neutrophils appear to require Vav1 and Vav3 for firm beta2-integrin-mediated adhesion but not for chemotaxis (130). In addition, Pyk2 inhibition in neutrophils diminishes integrin-dependent respiratory burst in response to TNF-alpha, while degranulation remains intact (131,132). In macrophages, Pyk2 deficiency results in defective chemotaxis, and these cells exhibit reduced PI3-kinase and Rho activation following integrin receptor engagement (133). In contrast to most other hematopoietic cell types studied, dendritic cell migratory speed, but not chemotaxis appears to be regulated partly by Pyk2 (134).

5.3. Rho-family GTPases regulate actin cytoskeletal dynamics downstream of integrin receptors

In all cell types, signaling through integrin receptors ultimately leads to the activation of Rho family GTPases and cytoskeletal reorganization. It is generally believed that a specific balance of GTP-loaded Rho GTPase family members and their subcellular localization determines the formation of particular actin cytoskeletal structures. Rac, Cdc42 and Rho are responsible for the formation of lamellipodia, fillipodia and stress fibers, respectively. During the early phases of integrin-mediated adhesion, Rho is less active and GTP-bound Rac and Cdc42 predominate; conditions that favor membrane protrusion (135). The downstream cytoskeletal effects of GTP-bound Rac and Cdc42 are mediated, at least in part, by a common effector PAK. PAK and its downstream effectors (e.g.: LIM kinase) promote actin filament assembly and stabilization by regulating the activity of the actin-depolymerizing protein cofilin/ADF. PAK also functions to decrease stress fiber formation by phosphorylating and inactivating MLC kinase, thereby preventing myosin-motor activity (136-138). Cdc42 exerts many of its effects on the actin cytoskeleton by promoting activation of the WASp/Arp2/3 actin-nucleating complex. GTP-bound Cdc42 binds to WASp-family proteins releasing them from an autoinhibitory conformation and unmasking the actin and Arp2/3 binding sites within the

WASp homology domain and C-terminal acidic region, respectively (139).

At later stages of adhesion activation of Rho appears to predominate, leading to the formation of stress fibers and focal adhesions that promote tension and firm adhesion (140). GTP-bound Rho has been shown to induce the assembly of actomyosin filaments and contractility both directly and through effectors such as ROCK (Rho-kinase) and mDia (141-143). Like the Rac/Cdc42 effector PAK, ROCK promotes filament stabilization by deactivating the actin-depolymerizing protein cofilin/ADF while mDia induces actin polymerization and regulates the organization of stress fibers (144,145).

In hematopoietic cells the role of Rho family GTPases in regulating integrin-mediated cytoskeletal changes is still not completely understood, and in some cases has been shown to vary based on the integrin substrate used (146). One example of this is the conflicting data regarding the function of Rho in regulating integrin-mediated adhesion in leukocytes, where both Rho activation and inhibition have been shown to promote cell spreading and/or adhesion (147-149). On the other hand, some integrin-mediated events such as rear-end detachment appear to be dependent on Rho activity in most cell types studied (150-152). Seemingly conflicting data have also been reported regarding the role of Rac in leukocytes. In a human T cell line overexpression of constitutively activated Rac diminished LFA-1-mediated spreading on ICAM-1 coated surfaces (153), while overexpression of activated Rac in a different T cell line promoted cell spreading and adhesion on fibronectin (154). Thus, hematopoietic cells likely rely on the coordinated activation of Rho-famly GTPases for regulating actin dynamics that release integrins from cytoskeleton constraint, promote clustering and increased avidity, and subsequent stabilization of the clustered conformation (focal contact). Still, a more precise understanding of the function of Rho-family GTPases in hematopoietic cells following integrin engagement will no doubt require additional studies comparing different cell types (e.g.; T cells versus dendritic cells) stimulated with multiple integrin agonists.

6. THE SYK PTK IS RECRUITED TO INTEGRIN BETA-CHAINS AND IS ACTIVATED FOLLOWING INTEGRIN LIGATION

6.1. Syk is an important mediator of integrin signaling in hematopoietic cells

The molecular mechanisms that govern "outside-in" integrin signaling have largely been characterized in cells of non-hematopoietic origin such as fibroblasts. Despite the identification of multiple molecular intermediates governing "outside-in" integrin signaling in non-hematopoietic cells, relatively little is known about the mechanisms by which integrins signal in hematopoietic cell types. Interestingly, hematopoietic cells differ in their cellular response to integrin ligation when compared to non-hematopoietic cells with respect to the actin-based structures that are formed. Hematopoietic cells such as leukocytes lack stress fibers and fail to form stable focal adhesions (reviewed in (144). Instead, leukocytes form relatively weak adhesions and are generally mobile or capable of obtaining a highly motile phenotype, unlike cells of non-hematopoietic origin (e.g. fibroblasts). This raises the interesting possibility that integrin signaling in hematopoietic cells may be governed by unique or cell-type specific signaling intermediates that confer unique adhesive and migratory capabilities upon hematopoietic cells. Unlike the TCR and some Fc receptors, integrin receptors do not contain ITAMs. Nonetheless, a growing body of evidence suggests that many of the same signaling intermediates that function downstream of ITAM-bearing antigen receptors are also required for optimal integrin signaling. The realization that Syk, a PTK expressed predominantly in hematopoietic cells, is recruited to integrin beta-chains and activated following integrin ligation in a number of hematopoietic cell types, suggested a whole new mechanism of integrin signaling in hematopoietic cells (155-160). Intriguingly, unlike the phosphotyrosine dependent association of Syk with ITAMS, Syk appears to be recruited to integrin beta-chains in a phosphotyrosine independent manner (161). However, as described for other receptor systems, Syk activation in hematopoietic cells following integrin stimulation requires Src PTK activity (162-165). Activation of the Syk PTK following integrin receptor engagement occurs upstream of actin polymerization and appears to be requisite for optimal integrin function in hematopoietic cells. Furthermore, truncation of the beta3- integrin cytoplasmic tail abolishes Syk activation while FAK phosphorylation is merely reduced, further supporting the existence of a specific integrin-induced Syk signaling pathway (159).

In macrophages and monocytes, Syk activity is required for changes in cell shape and spreading initiated by adhesion to ICAM-1 (166). In neutrophils, Syk is essential for beta2- integrin-mediated spreading and for respiratory burst induced by adhesion to a poly-RGD-coated surface. Other adhesion-dependent functions such as TNF-induced spreading and respiratory burst are also defective in Syk-/- neutrophils, while adhesion-independent functions such as TNF-induced CD18 upregulation and L-selectin shedding remain intact. Integrin-induced signaling events such as phosphorylation of downstream effectors like Vav, Pyk2 and ERK are also defective in neutrophils deficient for Syk (164). These data suggest that Syk is a critical mediator of integrin signaling in neutrophils but despite multiple defects in integrin-triggered signaling, Syk appears dispensable for normal neutrophil migration *in vitro* and *in vivo*. Furthermore, a slight increase in the migratory capacity of Syk deficient neutrophils or neutrophils lacking the Src kinase family members hck, fgr, and lyn was demonstrated in the presence of high chemokine concentrations (164). These data suggest that the defects in integrin receptor-mediated signaling in Syk or Src PTK deficient neutrophils may result in a low basal level of adhesion that actually promotes a slightly enhanced chemotactic potential. A similar phenotype has been observed in T cells that express a dominant negative form of the Syk family member ZAP-70. T cells do not normally express appreciable levels of Syk, and migration through ICAM-1-coated filters proceeds in the presence of

a dominant negative form of ZAP-70 only at high concentrations of chemokine and in an LFA-1 independent manner (167). Finally, a direct role for Syk in regulating the binding cycle of beta2- integrins in neutrophils has also been put forth. Interestingly, chemical inhibition of Syk in human neutrophils was actually shown to enhance adhesion to fibrinogen, but diminished adhesion to the same substrate when the cells were pre-treated with TNF-alpha. In addition, N-formyl-Met-Leu-Phe (FMLP)- induced chemotaxis was also defective following Syk inhibition perhaps due to the spontaneous and adhesion-induced enhancement of beta2-integrin clustering observed under these conditions (168).

In platelets, Src and Syk family PTKs also couple integrin engagement with downstream signaling events. Inhibition of Src PTKs or a deficiency of multiple Src kinase family members in platelets prevents adhesion-induced activation of Syk and phosphorylation of its substrates such as Vav1, Vav3 and SLP-76. These cells also spread poorly on fibrinogen, but this effect could be overcome by stimulation through G protein-coupled receptors. Syk deficient platelets exhibit similar defects in integrin function, including reduced spreading on fibrinogen that is also restored by G protein-coupled receptor signaling. Collectively, these data suggest that at least in platelets Syk is required for "outside-in" adhesion-induced spreading through integrins, but may be dispensable for adhesion-induced spreading mediated by G protein coupled receptor signaling (165).

6.2. SLP-76 and Vav are required signaling intermediates that couple integrin-mediated Syk activation with downstream signaling in hematopoietic cells

The demonstrated involvement of Syk in multiple integrin-triggered signaling events in platelets and neutrophils suggested that the downstream substrates of Syk may also function in these pathways and processes. Indeed, the Syk substrates Vav and SLP-76 are phosphorylated following integrin receptor engagement in a number of hematopoietic cell types and Vav or SLP-76 deficient platelets or neutrophils manifest a number of signaling defects following integrin ligation (91,124,163-165,169). As GEFs for Rho-family GTPases, Vav family members likely induce the activation of Rho GTPases following integrin simulation and help direct changes in the actin cytoskeleton that occur upon ligation of these receptors. This idea is supported by the observation that Vav1/3 double deficient neutrophils stimulated with TNF-alpha exhibited poor adhesion-induced activation of Rho, Rac and Cdc42 GTPases, defective phosphorylation of Pyk2 and almost a complete absence of PAK phosphorylation. The coexpression of Vav1 and Vav3 was also shown to be necessary for beta2-integrin-dependent functions such stable adhesion and spreading on ICAM-1 or fibrinogen-coated surfaces in response to inflammatory stimuli. Furthermore, the defects in integrin-mediated signaling and adhesion observed in Vav1/3 deficient neutrophils were most likely a result of impaired integrin signaling as chemotaxis and signaling in response to FMLP and leukotriene-B4 were unaltered in these cells, suggesting that G protein-coupled signaling was intact in these cells (130). In addition, the reduced integrin-mediated Pyk2 phosphorylation observed in the absence of both Vav1 and Vav3 indicates that Vav1/3 may influence Pyk2 activation by a currently unknown mechanism. In T cells, LFA-1-induced Rac activation was shown to be dependent on Vav activity (153). Perhaps related, adhesion dependent phosphorylation of Vav is reduced and correlates with diminished activation of the Rac/Cdc42 effector PAK in T cells treated with Src-PTK inhibitors (170). Although most reports suggest that Vav controls integrin receptor signaling events at the level of GTPase activation, a recent report has also described a novel role for Vav in integrin signaling in T cells independent of Vav's GEF activity. Overexpression of a constitutively active Vav mutant with Rac in T cells enhanced PAK activity and cell spreading upon integrin stimulation (171). The cooperative activity of Rac and Vav was observed even in the absence of Vav GEF activity implying that Vav may perform adaptor functions downstream of integrin receptors as well. Interestingly, this cooperation requires an intact Vav SH2 domain, the same domain that mediates Vav's association with SLP-76 following TCR stimulation.

The first direct evidence of SLP-76 involvement in integrin receptor-mediated signaling was demonstrated in an epithelial cell line stably expressing the platelet alpha(IIb)beta3 integrin receptor and transfected with SLP-76, Vav, and/or Syk (124). This study revealed Syk-dependent SLP-76 phosphorylation following alpha(IIb)beta3 -mediated binding to fibrinogen and the inducible formation of a complex containing SLP-76, Nck and Vav. In addition, adhesion to fibrinogen was increased in the presence of SLP-76, Vav and Syk as was the activation of the Rac/Cdc42 effector PAK. More recently, SLP-76 was also found to regulate integrin signaling in neutrophils. SLP-76 redistributes into punctate clusters at the plasma membrane and localizes at sites of substrate attachment upon neutrophil adhesion to beta1/3 and beta2-intergin agonists (91). In the absence of SLP-76, neutrophils fail to produce reactive oxygen intermediates or spread when plated on anti-CD18 or poly-RGD coated surfaces, ligands for beta1/3 and beta2 integrins respectively; a phenotype remarkably similar to that observed in Vav1/3 deficient neutrophils (130). Integrin-mediated signaling cascades such as Vav phosphorylation, PLCg2 and p38 MAPK activation were also defective in the absence of SLP-76 in neutrophils (91). Interestingly, these data also closely mimic the defects observed in Syk deficient neutrophils including decreased respiratory burst, spreading, Vav phosphorylation and p38 activation upon integrin engagement and further validate the importance of SLP-76 in propagating Syk-mediated integrin signals (164). Although not directly addressed in these studies, the poor induction of integrin-mediated Vav phosphorylation observed in the

absence of SLP-76 may be a result of defective Vav localization. Poor localization and activation of Vav may result in reduced Rho-family GTPase activation and could partially explain the spreading defect observed in SLP-76 deficient neutrophils.

Collectively, these important studies demonstrate that Syk, Vav, and SLP-76 function in multiple signaling pathways downstream of integrin ligation in hematopoietic cells, and suggest multiple immune cell-specific mechanisms whereby integrins couple with the actin cytoskeleton to regulate cell adhesion and motility. In addition to direct effects on Vav1/3 activity and function, how else might the Syk/SLP-76/Vav signaling axis impact cytoskeletal dynamics following integrin ligation? As discussed earlier, SLP-76 provides a scaffold for multiple proteins that modulate the cytoskeleton, including WASp. Thus, WASp/Arp2/3 mediated actin-polymerization is one example of a known signaling pathway that may be regulated by SLP-76 function in multiple hematopoietic cell types. Macrophages derived from WASp deficient patients fail to localize the Arp2/3 complex into cytoskeletal migratory structures and as a result, migrate poorly to a chemotactic stimulus (172,173). WASp deficient T cells and neutrophils exhibit decreased migration to a chemotactic stimulus as well, perhaps also due to defective Arp2/3 localization (174). Dendritic cells deficient for WASp exhibit decreased translocational mobility *in vitro* and recently, the *in vivo* migration of these cells was also found to be severely impaired (175,176). Interestingly, both WASp deficient macrophages and dendritic cells fail to form actin-based structures known as podosomes upon adhesion to surfaces coated with integrin ligands (172,177). Podosome expression is restricted to highly motile and/or invasive hematopoietic cells of the myeloid lineage such as macrophages, osteoclasts, and dendritic cells, but are also found in some virus-transformed fibroblasts and malignant B cells (reviewed in (178). Integrins and multiple cytoskeletal components (e.g. vinculin) have been shown to localize to these actin-rich structures. Currently, the precise function of podosomes is unclear but they most likely play a role in adhesion and may promote tissue invasion by directing metalloprotease localization and subsequent matrix degradation (178). Interestingly, our laboratory has made the recent observation that integrin-dependent adhesion and podosome distribution is markedly altered in SLP-76 deficient dendritic cells, perhaps due to poor WASP localization and/or activation (N. Luckashenak, unpublished observations).

6.3. Syk, SLP-76, and Vav may cooperate with Rap1/RAPL; potent regulators of integrin adhesion in the immune system

In 2003, Katagiri K *et al*. demonstrated that the Rap1-binding protein, RAPL is recruited to the membrane following TCR stimulation where it binds to and directs "outside-in" signaling to LFA-1 (179). The same group subsequently demonstrated that lymphocyte and dendritic cell adhesion and migration are also defective in RAPL deficient mice, implicating RAPL as an important mediator of "outside-in" integrin signaling as well (180). Currently, the mechanism by which RAPL regulates integrin-mediated adhesion and migration in hematopoietic cells is still somewhat of a mystery. Rap1 and RAPL may be linked to the cytoskeleton via a recently identified Rap1-binding protein RIAM (Rap1-GTP-interacting adaptor molecule), which has been shown to bind cytoskeletal proteins such as profilin and members of the Ena/VASP family (181). In addition, adhesion-induced membrane localization of RasGRP2, a Rap1-specific exchange factor is dependent on cytoskeletal reorganization and Vav-induced Rac activity (182). Given the defect in integrin-induced Vav phosphorylation in Syk and SLP-76 deficient neutrophils, it is possible that the localized activation of Rap1 at the cell membrane may also be sub-optimal in these cells. Defective Rap1 activation in Syk and SLP-76 deficient neutrophils might partially explain the integrin-mediated spreading defect observed in these cells.

7. SUMMARY AND PERSPECTIVE

The SLP-76 adaptor protein is clearly an integral component of multiple signaling pathways governing T lymphocyte activation, function, and development. The definition of SLP-76 dependent signaling pathways in T cells has provided an indispensable "roadmap" for beginning to identify how SLP-76 functions in a more recently appreciated role in propagating integrin signaling in hematopoietic cells. Defining the molecular mechanisms that govern "outside-in" signaling in hematopoietic cells is an area of great interest given the highly motile nature of many immune system cells and their dependence on adhesion and motility for proper function. Identification of Syk, SLP-76, and Vav1/3 as key regulators of integrin signaling in platelets and neutrophils suggests a mechanism for regulating actin polymerization that may be independent of or functioning in concert with the FAK family of adaptor/kinases. Until a few years ago, Syk expression was thought to be restricted to cells of hematopoietic origin. However, in recent years Syk expression has been observed in non-hematopoietic cells such as endothelial cells and mounting evidence indicates that Syk may regulate epithelial and endothelial anchorage-dependent growth (183). As discussed, multiple recent studies imply that Syk alone does not produce the hematopoietic cell type-specific responses observed following integrin engagement, and that additional proteins such as SLP-76 act in concert with Syk and the existing actin polymerizing machinery to mediate integrin-induced cytoskeletal dynamics. Therefore, it seems likely that hematopoietic cells employ at least two pathways that couple integrin signaling with changes in the cytoskeleton; the "classic" pathway involving Src-family PTKs and Fak, and the "hematopoietic" pathway, which relies on Syk, SLP-76, Vav and potentially Pyk2 to mediate downstream effects (Figure 2). Potential "cross-talk" between these two pathways is an area of investigation that may shed light

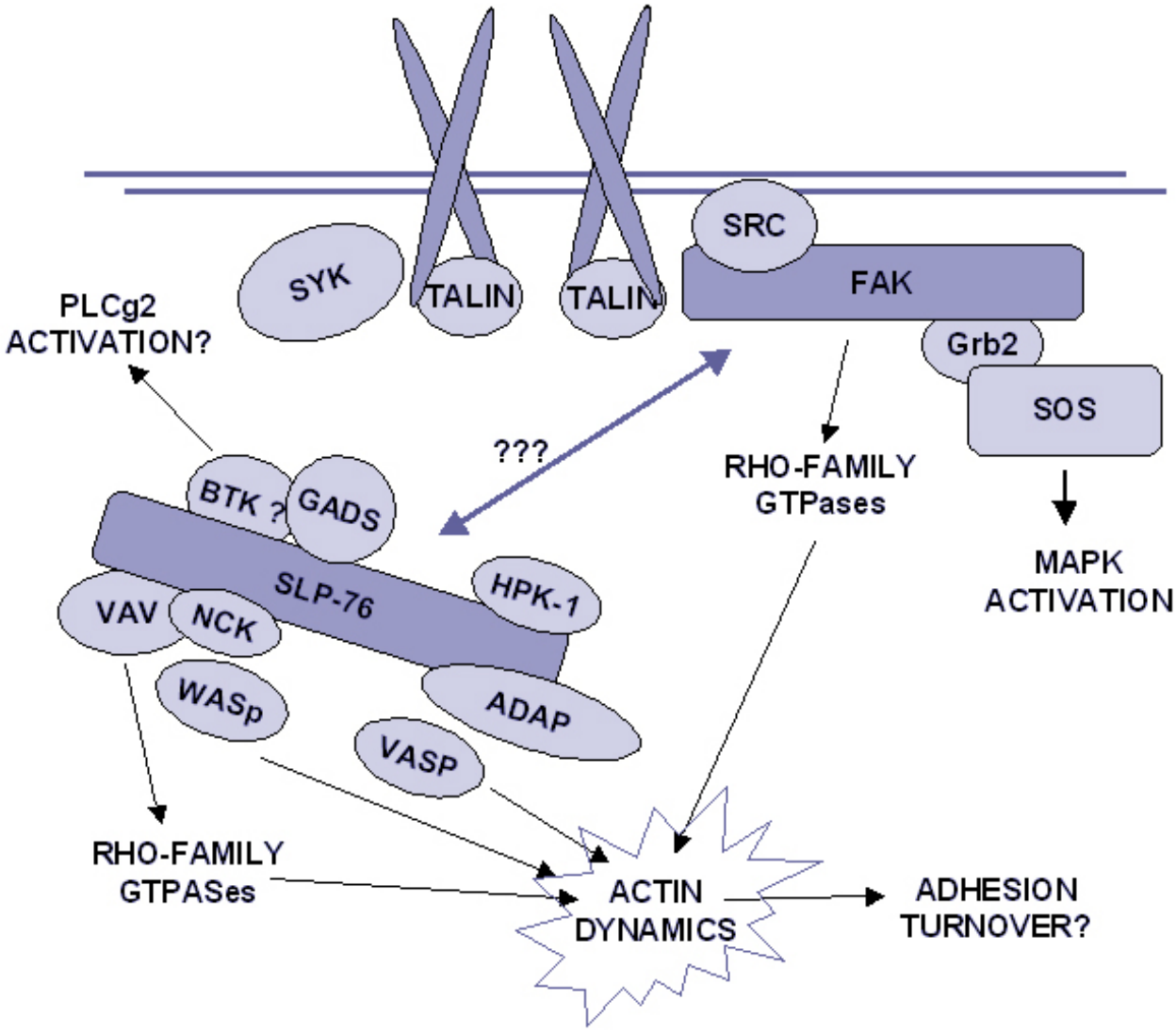

Figure 2. SLP-76 and FAK couple integrin ligation with more distal signaling events in hematopoietic cells. Following integrin ligation in hematopoietic cells, SLP-76 becomes phosphorylated on tyrosines and presumably recruits a profile of signaling intermediates similar to those described in T cells following TCR ligation (ie; Nck, Vav). While not proven experimentally, it seems likely that PLCg2 and the Itk homologue Btk may also be recruited to SLP-76 following integrin engagement. Likewise, if ADAP (and HPK-1?) is phosphorylated following integrin ligation, then ADAP should bind the SH2 domain of SLP-76. Thus, integrin engagement may promote the assembly of a SLP-76 nucleated multimolecular signaling complex similar in composition to that described in T cells following TCR ligation. Still, it remains possible that additional proteins distinct from those observed in T cells may be recruited to the SLP-76 complex following integrin ligation. It also remains to be determined if any "cross-talk" exists between SLP-76 and it's associated proteins and the FAK-dependent signaling axis in hematopoietic cells. Together, signaling events regulated by SLP-76 in combination with those governed by FAK may contribute to the unique structure and dynamics of actin-based adhesions in hematopoietic cells.

on how immune cells translate integrin ligation into the unique actin-based structures observed in these cells.

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Abbreviations: TCR: T cell receptor; ITAMs: immunoreceptor tyrosine-based activation motifs; ITIMs: immunoreceptor tyrosine-based inhibitory motifs; BCR: B cell receptor; PTKs: protein tyrosine kinases; SLP-76: SH2-domain containing leukocyte protein of 76 kD; LAT: linker for activation of T cells; BLNK: B cell-linker protein; APC: antigen presenting cell; MHC: major histocompatibility complex; MHC:p: MHC-peptide; IS: immunological synapse; cSMAC: central supramolecular activation cluster; pSMAC: peripheral supramolecular activation cluster; GEF: guanine nucleotide exchange factor; ERK: p44/42 MAP kinases; MAPK: map kinases; PAK: p21 activated kinase; PLCg: PLCgamma; ADAP: adhesion and degranulation promoting adaptor protein; WASp: Wiskott-Aldrich syndrome protein; MTOC: microtubule organization center; NK: natural killer; FAK: focal adhesion kinase; FERM: protein 4.1, ezrin, radixin and moesin homology; FAT: focal adhesion targeting; GAP: GTPase activating protein; mDia: mammalian diphanous; ROCK: Rho-kinase; MLC: myosin light chain; PIP_2: phosphatidylinositol (4,5)-bisphosphate; PH: pleckstrin homology; FMLP: N-formyl-Met-Leu-Phe; RIAM: Rap1-GTP-interacting adaptor molecule

Key Words: Antigen Receptor, TCR, SLP-76, Vav, ITAM, Syk, Integrin, Rho-Gtpase, Cytoskeleton, Signaling, Review

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