Constitutive Association of SHP-1 with Leukocyte-Associated Ig-Like Receptor-1 in Human T Cells

Jean G. Sathish, Kenneth G. Johnson, Kerensa J. Fuller, Frances G. LeRoy, Linde Meyaard, Martin J. Sims and R. James Matthews

_J Immunol_ 2001; 166:1763-1770; doi: 10.4049/jimmunol.166.3.1763

http://www.jimmunol.org/content/166/3/1763

References
This article cites 38 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/166/3/1763.full#ref-list-1

Subscription
Information about subscribing to _The Journal of Immunology_ is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Constitutive Association of SHP-1 with Leukocyte-Associated Ig-Like Receptor-1 in Human T Cells

Jean G. Sathish,* Kenneth G. Johnson,2* Kerensa J. Fuller, † Frances G. LeRoy,* Linde Meyaard, ‡ Martin J. Sims,3* and R. James Matthews* *

The intracellular Src homology 2 (SH2) domain-containing protein tyrosine phosphatase (SHP-1) is a negative regulator of cell signaling and contributes to the establishment of TCR signaling thresholds in both developing and mature T lymphocytes. Although there is much functional data implicating SHP-1 as a regulator of TCR signaling, the molecular basis for SHP-1 activation in T lymphocytes is poorly defined. A modification of the yeast two-hybrid system was employed to identify in T cells phosphotyrosine-containing proteins capable of binding the SH2 domains of SHP-1. From this yeast tri-hybrid screen, the p85β subunit of phosphatidylinositol 3-kinase and the immunoreceptor tyrosine-based inhibitory motif-containing receptors, leukocyte-associated Ig-like receptor-1 (LAIR-1), were identified. Coimmunoprecipitation studies demonstrated that the exclusive phosphotyrosine-containing protein associated with SHP-1 in Jurkat T cells under physiological conditions is LAIR-1. Significantly, this interaction is constitutive and was detected only in the membrane-enriched fraction of cell lysates. Ligand engagement of the SH2 domains of SHP-1 is a prerequisite to activation of the enzyme, and, consistent with an association with LAIR-1, SHP-1 was found to be constitutively active in unstimulated Jurkat T cells. Importantly, a constitutive interaction between LAIR-1 and SHP-1 was also detected in human primary T cells. These results illustrate the sustained recruitment and activation of SHP-1 at the plasma membrane of resting human T cells by an inhibitory receptor. We propose that this mechanism may exert a constitutive negative regulatory role upon T cell signaling. The Journal of Immunology, 2001, 166: 1763–1770.

1 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 This work was supported by a Wellcome Trust Research Career Development Fellowship and a Project Grant from the Wellcome Trust to R.J.M. J.G.S. is funded jointly by University of Wales College of Medicine and the Overseas Research Students’ Awards Scheme.

3 Address correspondence and reprint requests to Dr. R. James Matthews, Department of Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XX, Wales, U.K. E-mail address: matthewsrj@cardiff.ac.uk

4 Abbreviations used in this paper: SH2, Src homology 2; SHP, SH2 domain-containing protein tyrosine phosphatase; PTP, protein tyrosine phosphatase; LAIR-1, leukocyte-associated Ig-like receptor 1; ITIM, immunoreceptor tyrosine-based inhibitory motif; KIR, killer cell Ig-like receptor; MAPK, mitogen-activated protein kinase; PV, pervanadate.

Copyright © 2001 by The American Association of Immunologists
responsible for activating SHP-1 in Jurkat T cells has not been established.

It has been proposed that tyrosine phosphorylated ZAP-70 (9) or CD5 (7, 10) may satisfy the requirement for SHP-1 SH2 domain engagement and activation in Jurkat T cells. The results presented here now suggest that constitutively, tyrosine-phosphorylated LAIR-1 may in fact be the physiological ligand that allows the recruitment and activation of SHP-1 in Jurkat T cells. In addition, LAIR-1 is found constitutively associated with SHP-1 in human primary T cells. Together, these results suggest that the complex of LAIR-1 and SHP-1 may contribute to a basal threshold for TCR signaling in human T cells.

Materials and Methods

**Yeast tri-hybrid screen**

Yeast vector, pYTH9, and yeast strain, Ylkc, were as described in Ref. 17. Amino acids 1–222 of SHP-1 containing the two SH2 domains were cloned in frame into pYTH9, which contains amino acids 1–147 (the binding domain) of Gal4 protein to generate the bait construct pYTH9/ BDSHP1SH2. Strain Ylkc/BDSHP1SH2 was generated by integration of pYTH9/BDSHP1SH2 at the trpl locus of Ylkc. Transformants were cloned by restreaking for single clones on selective media. Recombinant yeast were maintained on selective media. Amino acids 728–864 of CD22 were cloned in-frame downstream of the activation domain of Gal4 in the pACT2 vector (Clontech Laboratories, Palo Alto, CA) to serve as a positive control.

All yeast manipulations were performed according to standard yeast genetic protocols. Competent yeast cells were prepared and transformed using the lithium acetate/Tris-EDTA/polyethylene glycol protocol (17). The cDNA library was donated by M. Suda of GlaxoNippon (Tsukuba, Japan). The cDNA was generated from poly(A)+ RNA extracted from a mixture of nonactivated and 12-O-tetradecanoylphorbol-13-acetate-activated H9 3D8 cells and synthesized using an oligo(dT) primer and random primers. cDNA was unidirectionally cloned between the EcoRI and Xhol sites of pACT2. CDNA library screening was performed using Ylkc/ BDSHP1SH2. Approximately 1 × 10⁶ cells were transformed with 50 μg of H9 library DNA and plated onto minimal agar containing yeast nitrogen base, 2% glucose, and 5 mM 3-amino-1,2,4-triazole, a competitive inhibitor of the HIS3 gene, one of the reporter genes in this strain. Transformation plates were incubated at 30°C for 10–14 days. Large colonies were selected from these plates and streaked onto smaller plates with or without methionine but supplemented with histidine. These were left to grow for 3 days and then filter-lifted and screened for protein–protein interactions using the EcoRI and Xhol sites of pACT2. CDNA library screening was performed using Ylkc/ BDSHP1SH2. Strain Ylkc/BDSHP1SH2 was generated by integration of pYTH9/BDSHP1SH2 at the trpl locus of Ylkc. Transformants were cloned by restreaking for single clones on selective media. Recombinant yeast were maintained on selective media. Amino acids 728–864 of CD22 were cloned in-frame downstream of the activation domain of Gal4 in the pACT2 vector (Clontech Laboratories, Palo Alto, CA) to serve as a positive control.

**Subcellular fractionation**

A total of 0.5–1.0 × 10⁶ Jurkat T cells were left unstimulated or stimulated with either 10 μg/ml UCHT-1 for 3 min at 37°C or with 200 μM pervanadate (PV) for 10 min at 37°C. Following stimulation, cells were pelleted and resuspended in 2 ml ice-cold hypotonic lysis buffer (10 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 1 mM sodium orthovanadate) containing protease inhibitors (10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 1 mM EDTA, 1 mM PMSF) added immediately before use. After 30 min incubation on ice, lysis was facilitated by Dounce homogenization for 12 strokes on ice, and lysates were clarified to remove nuclear material and intact cells. Supernatants were transferred into ultracentrifugation tubes (Beckman Coulter, Fullerton, CA), and fractionation was performed at 100,000 × g for 30 min at 4°C (TL-100 Ultracentrifuge; Beckman Coulter). The supernatant containing the solubilized (S100) fraction was removed, and NaCl and Nonidet P-40 were added to a final concentration of 150 mM and 0.5%, respectively. The pellet constituting the particulate (P100) fraction was solubilized in 1 ml Nonidet P-40 lysis buffer containing protease inhibitors as above.

**Immunoprecipitation and immunoblot analysis**

Prepared cell lysates were incubated by continuous inversion for 1 h at 4°C with either purified monoclonal or polyclonal Abs. Immune complexes were trapped by rotating at 4°C for a further hour with the addition of protein A or protein G Sepharose slurry. GST fusion proteins immobilized on glutathione-agarose beads were similarly incubated with lysates for 90 min at a concentration of 20 μg/ml. Captured complexes were pelleted by centrifugation and washed in 0.5% Nonidet P-40 lysis buffer an additional two times. Following washes, beads were resuspended in 2× Laemmli reducing buffer or 2× Laemmli nonreducing buffer for samples to be immunoblotted with the anti-LAIR-1 mAb, DX26. Immunoprecipitations and cell lysates were resolved by 12% SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA), and probed with specific Abs followed by HRP-conjugated secondary Abs. Proteins were detected by ECL (Amersham Pharmacia Biotech, Piscataway, NJ). Blots were stripped for 30 min at 55°C (100 mM 2-ME, 2% SDS, 62.5 mM Tris-HCl (pH 6.8)) before reprobing.

**PTP activity assay**

SHP-1 was immunoprecipitated from the particulate (P100) and cytosolic (S100) fractions of unstimulated or CD3-stimulated Jurkat T cells as described above. Immunoprecipitates were assayed for PTP activity using 32P-labeled Roytide as described in Ref. 1. PTP activities in the immunoprecipitates were normalized by performing densitometry on the amount of SHP-1 detected in parallel anti-SHP-1 immunoprecipitations. Normalizations were further confirmed by performing serial...
the gradient and solubilized in 6 M Laemmli nonreducing buffer.

Inverted at 200,000 g for 18 h at 4°C and centrifuged for 18 h at 4°C at 200,000 × g. Then, 0.4-ml fractions were collected from the top of the gradient and solubilized in 6× Laemmli nonreducing buffer.

**IL-2 release assay**

Jurkat T cells were stimulated in triplicate on 96-well flat-bottom plates at 2 × 10⁵ cells/well with a titration of bi-specific Frab (1:2), CD3 × CD28 Ab in the presence of LAIR-1 (DX26) or control IgG1Ab (5 μg/ml) cross-linked with a rabbit anti-mouse Ab (10 μg/ml). After 6 h, the supernatants were harvested. For a measure of IL-2 production, extensively washed CTLL-2 cells were added to diluted supernatants (1:2) and incubated for 40 h with [3H]thymidine (1 Ci/well) present for the last 16 h of culture before harvesting, and the incorporated radioactivity was assessed.

**Results**

**Interaction of the SH2 domains of SHP-1 and ITIM-containting proteins in a yeast tri-hybrid screen**

The identities of phosphotyrosine-containing proteins capable of interacting with the SH2 domains of SHP-1 in T cells have not been readily forthcoming. Therefore, we modified a known cDNA cloning strategy, the yeast two-hybrid, to create a yeast tri-hybrid (17) to identify, from T cells, those phosphotyrosine proteins capable of interacting with the SH2 domains of SHP-1. A stable yeast strain, Ylck/BDSHP1SH2, was generated expressing both the active form of the protein tyrosine kinase lck under a regulatable promoter and a chimeric cDNA encoding the tandem SH2 domains (amino acids 1–222) (1) of SHP-1 fused to the DNA binding domain of Gal4. The regulatable expression of lck in the recombinant yeast via the MET3 promoter allowed for the identification of interactions that were dependent on the tyrosine phosphorylation of either bait or prey proteins (17). The validity of the yeast tri-hybrid system for identifying ligands of the SH2 domains of SHP-1 was established using CD22 as a positive control (results not shown). Ylck/BDSHP1SH2 was transformed with a cDNA library derived from the H9 T cell line. The cDNAs from positive clones were isolated and sequenced, and three proteins were identified (Fig. 1). They were PD-1 (one clone encoding amino acids 226–289 and encompassing part of the intracellular domain and only one ITIM of the two possible ITIMs contained in this protein), LAIR-1 (one clone encoding amino acids 56–288 and encompassing most of the extracellular domain, the transmembrane domain, and all of the intracellular region including both ITIMs of this protein), and the β-type 85-kDa subunit (p85β) of phosphatidylinositol 3-kinase (two clones encoding amino acids 64–729 and 321–729, with no consensus ITIM motifs contained within these proteins).

The interaction of the SH2 domains of SHP-1 with each of the ligands was only detected in the absence of methionine and was therefore dependent on expression of lck. To test the dependence of the tri-hybrid interactions on the SH2 domains of SHP-1, mutations at critical arginine residues in the SHP-1 SH2 domains (R30K and R136K) (18, 21) were used. The transformation of Ylck with the BDSHP1SH2R30K/R136K mutant construct and each of the three ligands demonstrated the critical requirement of at least one intact SH2 domain for the formation of the SHP-1 (amino acids 1–222) fusion protein and each of the three ligands (Fig. 1). In addition, as demonstrated in yeast strain Ylck/BDSHP1SH2R30K, an intact amino-terminal SH2 domain of SHP-1 appeared to be critically required for the physical association of the SHP-1 (amino acids 1–222) chimera with LAIR-1 and p85β but not PD-1 (Fig. 1).

**Catalytically active SHP-1 is constitutively associated with the membrane fraction of Jurkat T cells**

Having demonstrated that the SH2 domains of SHP-1 could interact with the intracytoplasmic domains of LAIR-1 and PD-1 and with p85β in yeast, we were interested in establishing whether any of these interactions could be detected in T cells. First, LAIR-1 and PD-1 are transmembrane receptors, and we reasoned that any
physiological associations with SHP-1 would be occurring predominantly at the cell membrane. Therefore, Jurkat T cells were subjected to subcellular fractionation to generate membrane (P100) and cytosolic (S100) fractions. The validity of the fractionation procedure was evaluated by immunoblotting with anti-CD3ε and MAPK Abs (Fig. 2A). As previously reported for other leukocytes, over 90% of SHP-1 was localized to the cytosolic fraction (22). However, 5–10% of SHP-1 was found constitutively associated with the P100 fraction in Jurkat T cells (Fig. 2A). The amount of SHP-1 in the P100 fraction did not significantly alter upon anti-CD3 or PV treatment (Fig. 2A). To examine the catalytic activity of SHP-1 in the P100 fraction of Jurkat T cells, SHP-1 was immunoprecipitated from the P100 and S100 fractions of unstimulated or anti-CD3-stimulated Jurkat T cells, and the PTP activity was measured (Fig. 2B). The catalytic activity of SHP-1 in the P100 fraction was found to be 2- to 3-fold higher than that of SHP-1 in the S100 fraction. It is believed that the PTP activity of SHP-1 is liberated through the occupation of the amino-terminal SH2 domain by phosphotyrosine-containing ligands. Therefore, we sought to identify the phosphotyrosine-containing protein(s) responsible for activating SHP-1 within the membrane fraction.

**FIGURE 2.** Constitutively active SHP-1 is found associated with the membrane fraction of unstimulated Jurkat T cells. Jurkat T cells remained unstimulated or were stimulated with anti-CD3 or PV, lysed in hypotonic lysis buffer, and subjected to subcellular fractionation. A, Lysates from the membrane (P100) and cytosolic (S100) fractions of either resting or stimulated Jurkat T cells were separated by SDS-PAGE and subjected to immunoblotting with either anti-CD3ε or anti-MAPK as controls for the P100 and S100 fractions, respectively, or with anti-SHP-1. B, Lysates from the membrane (P100) and cytosolic (S100) fractions of either resting or stimulated Jurkat T cells were subjected to immunoprecipitation with preimmune or anti-SHP-1 sera. PTP activities in the immunoprecipitates were measured using a 32P-labeled Raytide peptide as a substrate and normalized to the amount of SHP-1 detected by immunoblotting of each immunoprecipitate. PTP activities are shown as the amount of radioactivity (cpm) released above background from the 32P-labeled peptide.

SHP-1 was immunoprecipitated from the P100 and S100 fractions of unstimulated anti-CD3 or PV-stimulated Jurkat T cells, and the immunoprecipitates were analyzed by anti-phosphotyrosine immunoblotting (Fig. 3). Within the P100 fractions, SHP-1 only immunoprecipitated a 42-kDa phosphotyrosine-containing protein (pp42). The 26-kDa phosphotyrosine-containing protein found in the P100 fraction likely represents CD3ε that is immunoprecipitated by the stimulating Ab because it was detected in both control and anti-SHP-1 immunoprecipitations following anti-CD3 activation. Furthermore, the preclearing of the lysates resulted in the disappearance of the 26-kDa phosphotyrosine-containing protein (results not shown). No phosphotyrosine proteins were found to coinmunoprecipitate with SHP-1 in the S100 fraction, although SHP-1 itself was constitutively tyrosine phosphorylated in this fraction (Fig. 3, and results not shown). Significantly, SHP-1 was constitutively associated with pp42 in the P100 fraction of unstimulated Jurkat T cells (Fig. 3). However, anti-CD3 stimulation resulted in an apparent decrease in the tyrosine phosphorylation of pp42 associated with SHP-1 in the P100 fraction. Parallel immunoprecipitations with SHP-1 and SHP-2 antisera from the P100 fraction demonstrated the constitutive association of pp42 with SHP-1 but not SHP-2 (Fig. 4A). The SH2 domains of SHP-1 mediated the association of pp42 with SHP-1. Lysates from the particulate fraction of unstimulated Jurkat T cells were absorbed onto GST or a GST fusion protein encoding the tandem SH2 domains of SHP-1. The tandem SH2 domains of SHP-1 (Fig. 4B) specifically absorbed pp42.

**pp42 is identified as LAIR-1**

The SDS-PAGE migration properties of pp42 associating with SHP-1 in Jurkat T cells resembled that of the ITIM-containing receptor, LAIR-1. The identity of pp42 as LAIR-1 was confirmed by immunoblotting SHP-1 immunoprecipitations from P100 fractions with LAIR-1 antiserum (Fig. 5A). Immunoprecipitation of LAIR-1 followed by anti-phosphotyrosine immunoblotting confirmed that LAIR-1 was constitutively tyrosine phosphorylated in the membrane fraction of unstimulated Jurkat T cells (Fig. 5B). In contrast to the decrease in the tyrosine phosphorylation of pp42 associated with SHP-1 (Fig. 3), anti-CD3 treatment of Jurkat T cells resulted in no change in the amount of LAIR-1 associated with SHP-1 (Fig. 5A). In addition, anti-CD3 stimulation had no effect on the basal level of tyrosine phosphorylation of LAIR-1 (Fig. 5B). In parallel experiments, stimulation of Jurkat T cells with soluble anti-CD3ε (UCHT-1) could be demonstrated to result in an extensive tyrosine phosphorylation of the CD3 chains and downstream signaling mediators (results not shown).

**LAIR-1 is found in a saponin-sensitive membrane compartment**

To understand the mechanism of how LAIR-1 is constitutively tyrosine phosphorylated, we hypothesized that LAIR-1 could be localized in a specialized subcompartment in the membrane that allowed it access to an active protein tyrosine kinase under basal conditions. One method for separation of membrane subcompartments based on their glycosphingolipid and cholesterol content is sucrose density gradient fractionation following lysis of cells in Triton X-100 at 4°C (23). Saponin treatment can be used to identify cholesterol-enriched membrane fractions by depleting cholesterol and rendering them soluble in Triton X-100 (24). Jurkat T cells were left untreated or treated with saponin, extracted with Triton X-100, and fractionated on a sucrose gradient (Fig. 6). Among the 12 fractions collected, fractions 2 and 3 contained the
classic low-sucrose-density detergent-insoluble glycosphingolipid-enriched domain (25), as demonstrated by the localization of p56\.lck in these fractions (26). Fractions 8–12 from the gradient represented the Triton X-100 soluble fractions. Interestingly, LAIR-1 was found excluded from the low-sucrose-density, detergent-insoluble glycosphingolipid-enriched domain-containing membrane compartment, but was found localized in part to a saponin-sensitive, intermediate-sucrose-density membrane compartment (fraction 7) (Fig. 6). As a negative control, CD45 localized to the high-sucrose-density membrane compartment (27) that remained insensitive to saponin treatment.

LAIR-1 cross-linking has no effect on the threshold of TCR signaling

LAIR-1 possesses a single Ig-like domain in its extracellular region (15), suggesting the existence of a cell surface ligand that would potentially regulate the localization of LAIR-1 in the membrane. A ligand for LAIR-1 could potentially either cluster LAIR-1 away from the TCR or recruit LAIR-1 to the TCR, thereby lowering or raising the threshold for TCR signaling, respectively. In addition, a ligand could regulate the tyrosine phosphorylation of LAIR-1 and consequently determine SHP-1 recruitment to the cell membrane. In the absence of an identified ligand for LAIR-1, we used the anti-LAIR-1 mAb, DX26 (15), to cross-link LAIR-1, in conjunction with a soluble bi-specific Ab directed against CD3 and CD28, and examined the effects on IL-2 production. No significant differences in IL-2 production in response to CD3 and CD28 cross-linking were detected as a result of the simultaneous cross-linking of LAIR-1 on Jurkat T cells (Fig. 7). The direct co-cross-linking of LAIR-1 and CD3 also had no effect on IL-2 production in Jurkat T cells (results not shown).

LAIR-1 is found constitutively associated with SHP-1 in human primary T cells

Having detected a constitutive interaction between SHP-1 and LAIR-1 in human transformed T cells, we investigated whether a similar association occurred in human primary T cells. In this regard, LAIR-1 was immunoprecipitated from T lymphoblasts with the anti-LAIR-1 mAb, DX26 (15), and was demonstrated to be constitutively associated with SHP-1 (Fig. 8). Anti-CD3 stimulation resulted in a detectable increase in the amount of SHP-1 found associated with LAIR-1 (Fig. 8). No SHP-1 could be detected co-immunoprecipitating with an isotype-matched control for DX26 (result not shown). In contrast to Jurkat T cells, the basal and anti-CD3-stimulated level of tyrosine phosphorylation of LAIR-1 was below a level of detection in the T lymphoblasts, and only upon PV treatment did LAIR-1 tyrosine phosphorylation become
apparent. As with Jurkat T cells, anti-LAIR-1 cross-linking with the mAb DX26 on T lymphoblasts had no effect on the basal LAIR-1/SHP-1 association (results not shown).

Discussion

This study describes an important constitutive association between SHP-1 and the ITIM-containing receptor, LAIR-1, in human T cells. As an initial strategy for identifying potential T cell-derived ligands of the SH2 domains of SHP-1, a modified yeast two-hybrid screen was developed. By using integrated plasmids, our system allows the generation of stable yeast strains, which facilitates the recovery of prey plasmids. In addition, the regulatable kinase allows phosphotyrosine-dependent interactions to be readily distinguished. ITIMs have been well documented as providing high-affinity binding sites for the SH2 domains of SHP-1. The intracytoplasmic regions of PD-1 (28) and LAIR-1 (15) isolated in the tri-hybrid screen possess one and two ITIMs, respectively. It thus appears that in the context of the yeast tri-hybrid system, a single ITIM on a ligand may be sufficient for a detectable interaction with the SH2 domains of SHP-1. Interestingly, p85β does not possess any ITIMs, and so the molecular basis of the interaction between p85β and the SH2 domains of SHP-1 in the yeast requires further analysis.

Having demonstrated that PD-1, LAIR-1, and p85 can interact with the SH2 domains of SHP-1 in yeast, it was important to demonstrate physiological interactions in T cells. We initially focused on the transformed T cell line Jurkat because functional data implicating SHP-1 in regulating thresholds of TCR activation have been obtained using this line (9). However, we were unable to detect significant coimmunoprecipitation between SHP-1 and any phosphotyrosine-containing proteins in unstimulated or anti-CD3-stimulated Jurkat cell detergent lysates. Therefore, although phosphotyrosine-containing ligand(s) of SHP-1 must exist in Jurkat T cells in lieu of previously described functional data (9), these ligand-SHP-1 complexes must occur at very low levels.

We detected no interaction between tyrosine-phosphorylated p85α or β and SHP-1 in Jurkat T cells under any conditions. Therefore, the physiological significance of the interaction of the SH2 domains of SHP-1 and tyrosine-phosphorylated p85 in the yeast remains to be determined. Likewise, we were unable to detect an interaction of SHP-1 and a tyrosine-phosphorylated molecule of the approximate molecular mass of PD-1 (31 kDa) in Jurkat T cells (29). For this reason, PD-1 is unlikely to be the ligand implicated in the activation of SHP-1 in Jurkat T cells. Instead, the major physiological phosphotyrosine-containing ligand of SHP-1 in Jurkat T cells is identified as LAIR-1. Significantly, this interaction was demonstrated to be constitutive, and therefore it is likely that the constitutive LAIR-1 interaction with SHP-1 accounts for the hyperresponsiveness of TCR signaling following the introduction of catalytically inactive SHP-1 into Jurkat T cells (9). Importantly, the constitutive interaction of LAIR-1 and SHP-1 is not an exclusive feature of Jurkat T cells, but can also be detected in primary human T cells.

The effects of anti-CD3 stimulation on the interaction of LAIR-1 and SHP-1 in Jurkat T cells and T lymphoblasts were distinct. In

FIGURE 6. LAIR-1 is found in a saponin-sensitive membrane compartment of Jurkat T cells. Jurkat T cells were left untreated or treated with 0.2% saponin, lysed in ice-cold 1% Triton X-100, and fractionated on a 5–40% sucrose density gradient. Following centrifugation, 12 sequential fractions were removed from the top of the gradient and mixed with 6X nonreducing Laemmli buffer. Aliquots from each fraction were separated by SDS-PAGE, and the distribution of LAIR-1, p56kcs, and CD45 in the different fractions of the sucrose gradient was revealed by specific immunoblotting.

FIGURE 7. Cross-linking of LAIR-1 has no effect on IL-2 production in CD3 × CD28-stimulated Jurkat T cells. Jurkat T cells were stimulated for 6 h with soluble bi-specific anti-CD3 × CD28 Ab in the presence or absence of cross-linked control or anti-LAIR-1 mAb, DX26 (5.0 μg/ml). IL-2 released into the culture supernatants was assayed as a function of [3H]hymidine incorporation in CTLL-2 cells. Shown is the radioactivity incorporated in CTLL-2 cells.

FIGURE 8. Constitutive association between LAIR-1 and SHP-1 in human primary T cells. T lymphoblasts were left untreated or stimulated with either anti-CD3 or PV, lysed in detergent buffer, subjected to immunoprecipitation with the anti-LAIR-1 mAb, DX26, and immunoblotted for either SHP-1 or phosphotyrosine.
Jurkat T cells, the triggering of CD3 led to no increases in the basal association of LAIR-1 and SHP-1. Paradoxically, the tyrosine phosphorylation of LAIR-1 that specifically coimmunoprecipitated with SHP-1 was reproducibly decreased in multiple experiments following anti-CD3-mediated activation. This decrease in phosphorylation of LAIR-1 appears to be limited to that bound to SHP-1 because no detectable change in total LAIR-1 phosphorylation occurs. It is possible that anti-CD3 stimulation induces a dephosphorylation of one of the ITIMs of LAIR-1 that is associated with SHP-1. However, it is conceivable that although both ITIM motifs in LAIR-1 may be necessary for the initial recruitment of SHP-1, once SHP-1 has bound LAIR-1, the dephosphorylation of one of the ITIMs on LAIR-1 may not cause a significant loss of SHP-1 binding. In contrast, the triggering of CD3 on T lymphoblasts results in a small increase in the amount of SHP-1 associated with LAIR-1, despite our inability to detect increases in the phosphotyrosine content of LAIR-1. We assume that the basal difference in the intensity of LAIR-1 tyrosine phosphorylation in Jurkat vs primary T cells is a consequence of a higher protein tyrosine kinase activity in the transformed T cells. Nevertheless, the key phenomenon of a constitutive association between LAIR-1 and SHP-1 is conserved in both primary and transformed T cells.

The constitutive tyrosine phosphorylation of LAIR-1 in human T cells illustrates a recurrent feature of ITIM-containing receptors. Both CD22 (30) on primary splenic B cells, and paired Ig-like receptor B on bone marrow-derived macrophages (31, 32) and primary splenic B cells (33) have been demonstrated to be constitutively tyrosine phosphorylated. A low level of constitutive tyrosine phosphorylation would enable ITIM-containing receptors to contribute to the establishment of basal thresholds of activation. The examination of thymocytes and peripheral T cells from SHP-1-deficient mice has provided substantial evidence to implicate SHP-1 in regulating the TCR signaling thresholds that govern T cell development and the activation of mature T cells (3–5, 7, 34). However, the molecular basis for SHP-1 activation in mouse thymocytes and peripheral T cells still remains to be fully elucidated. With regard to the constitutive activation of SHP-1 human T cells, it is particularly intriguing that the phenotype of motheaten lymph node T cells expressing the transgenic TCR, F5, suggests that SHP-1 is constitutively active in resting primary lymph node T cells (4). Thus far, there have been no reports of a mouse homologue of LAIR-1, and hence it is possible that one or more additional members of the family of ITIM-containing receptors are responsible for SHP-1 activation in mouse thymocytes and peripheral T cells.

Src-like kinases have been implicated in the PV-mediated phosphorylation of LAIR-1 in Jurkat T cells (35) and may presumably mediate the basal tyrosine phosphorylation of LAIR-1. Interestingly, p56Lck isolated from low-sucrose-density membrane fractions has been demonstrated to possess low kinase activity (27), although full TCR signaling function nevertheless requires the S-acylation of p56Lck (36). Therefore, it is conceivable that active p56Lck may be in a region of the membrane corresponding to the saponin-sensitive, intermediate-sucrose-density membrane fraction. Furthermore, the localization of a subpool of LAIR-1 to the intermediate-sucrose-density membrane compartment in unstimulated Jurkat may thereby expose it to active p56Lck.

The basal phosphorylation of LAIR-1 leading to constitutive activation of SHP-1 suggests that SHP-1 may dephosphorylate the TCR complex or a downstream component of the TCR signaling pathway, thereby contributing to the generation of a basal threshold of activation of T cells. However, it is not apparent how a LAIR-1-SHP-1 complex would gain access to the TCR or TCR signaling pathway. Nevertheless, it would be predicted from the results with catalytically inactive SHP-1 (9) that a LAIR-1-SHP-1 complex is able to influence the outcome of TCR signaling without a requirement for specific coengagement of LAIR-1 and TCR. Similarly, the introduction of catalytically inactive SHP-1 by transfection into mouse T cell hybridoma lines (3, 9) or by transgenesis into primary thymocytes (5) results in an increased sensitivity to anti-CD3 stimulation, without a requirement for the coengagement of any additional receptors. In addition, expression of a HLA-A2/SHP-1 chimera in Jurkat T cells requires no specific coligation with the TCR to be able to negatively affect anti-CD3-triggered signaling (37). Finally, the MHC class I engagement of a KIR on a CD4+ T cell clone expressing a MHC class II-restricted TCR is sufficient to regulate cytokine production elicited by bacterial superantigen (38). Taken together, these results suggest that the recruitment of SHP-1 to the plasma membrane, without a direct physical juxtaposition to the TCR, may be sufficient to influence the outcome of TCR signaling.

LAIR-1 possesses a single Ig-like domain in its extracellular region, suggesting the possible existence of a cell surface ligand. In an attempt to mimic the potential effects of LAIR-1 ligand engagement, independent cross-linking of LAIR-1 or simultaneous co-cross-linking with CD3 on Jurkat T cells was performed. Under both conditions, anti-CD3-induced production of IL-2 was unaffected. Furthermore, in keeping with these functional observations, the direct cross-linking of LAIR-1 with DX26 on Jurkat or primary T cells resulted in no increase in either the phosphotyrosine content of LAIR-1 or SHP-1 recruitment. However, the recent demonstration that treatment of Jurkat T cells with a polyclonal serum against LAIR-1 results in no increase in either the phosphotyrosine content of LAIR-1 or SHP-1 binding. Nevertheless, it is conceivable that although both ITIM motifs in LAIR-1 may be necessary for the initial recruitment of SHP-1, once SHP-1 has bound LAIR-1, the dephosphorylation of one of the ITIMs on LAIR-1 may not cause a significant loss of SHP-1 binding. In contrast, the triggering of CD3 on T lymphoblasts results in a small increase in the amount of SHP-1 associated with LAIR-1, despite our inability to detect increases in the phosphotyrosine content of LAIR-1. We assume that the basal difference in the intensity of LAIR-1 tyrosine phosphorylation in Jurkat vs primary T cells is a consequence of a higher protein tyrosine kinase activity in the transformed T cells. Nevertheless, the key phenomenon of a constitutive association between LAIR-1 and SHP-1 is conserved in both primary and transformed T cells.

The constitutive tyrosine phosphorylation of LAIR-1 in human T cells illustrates a recurrent feature of ITIM-containing receptors. Both CD22 (30) on primary splenic B cells, and paired Ig-like receptor B on bone marrow-derived macrophages (31, 32) and primary splenic B cells (33) have been demonstrated to be constitutively tyrosine phosphorylated. A low level of constitutive tyrosine phosphorylation would enable ITIM-containing receptors to contribute to the establishment of basal thresholds of activation. The examination of thymocytes and peripheral T cells from SHP-1-deficient mice has provided substantial evidence to implicate SHP-1 in regulating the TCR signaling thresholds that govern T cell development and the activation of mature T cells (3–5, 7, 34). However, the molecular basis for SHP-1 activation in mouse thymocytes and peripheral T cells still remains to be fully elucidated. With regard to the constitutive activation of SHP-1 human T cells, it is particularly intriguing that the phenotype of motheaten lymph node T cells expressing the transgenic TCR, F5, suggests that SHP-1 is constitutively active in resting primary lymph node T cells (4). Thus far, there have been no reports of a mouse homologue of LAIR-1, and hence it is possible that one or more additional members of the family of ITIM-containing receptors are responsible for SHP-1 activation in mouse thymocytes and peripheral T cells.

Src-like kinases have been implicated in the PV-mediated phosphorylation of LAIR-1 in Jurkat T cells (35) and may presumably mediate the basal tyrosine phosphorylation of LAIR-1. Interestingly, p56Lck isolated from low-sucrose-density membrane fractions has been demonstrated to possess low kinase activity (27), although full TCR signaling function nevertheless requires the S-acylation of p56Lck (36). Therefore, it is conceivable that active p56Lck may be in a region of the membrane corresponding to the saponin-sensitive, intermediate-sucrose-density membrane fraction. Furthermore, the localization of a subpool of LAIR-1 to the intermediate-sucrose-density membrane compartment in unstimulated Jurkat may thereby expose it to active p56Lck.

The basal phosphorylation of LAIR-1 leading to constitutive activation of SHP-1 suggests that SHP-1 may dephosphorylate the TCR complex or a downstream component of the TCR signaling pathway, thereby contributing to the generation of a basal threshold of activation of T cells. However, it is not apparent how a LAIR-1-SHP-1 complex would gain access to the TCR or TCR signaling pathway. Nevertheless, it would be predicted from the results with catalytically inactive SHP-1 (9) that a LAIR-1-SHP-1 complex is able to influence the outcome of TCR signaling without a requirement for specific coengagement of LAIR-1 and TCR. Similarly, the introduction of catalytically inactive SHP-1 by transfection into mouse T cell hybridoma lines (3, 9) or by transgenesis into primary thymocytes (5) results in an increased sensitivity to anti-CD3 stimulation, without a requirement for the coengagement of any additional receptors. In addition, expression of a HLA-A2/SHP-1 chimera in Jurkat T cells requires no specific coligation with the TCR to be able to negatively affect anti-CD3-triggered signaling (37). Finally, the MHC class I engagement of a KIR on a CD4+ T cell clone expressing a MHC class II-restricted TCR is sufficient to regulate cytokine production elicited by bacterial superantigen (38). Taken together, these results suggest that the recruitment of SHP-1 to the plasma membrane, without a direct physical juxtaposition to the TCR, may be sufficient to influence the outcome of TCR signaling.

Acknowledgments

We thank Dr. Joseph Phillips for the original provision of the anti-LAIR-1 mAb, DX26, and Prof. Doug Fearon for the CD22 cDNA. We thank colleagues M. Labeta, P. Brennan, and H. Clevers for advice and critical comments on the manuscript.

References


