CD22 Is a Functional Ligand for SH2 Domain-containing Protein-tyrosine Phosphatase-1 in Primary T Cells*

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The intracellular Src homology 2 (SH2) domain-containing protein-tyrosine phosphatase (SHP-1) has been characterized as a negative regulator of T cell function, contributing to the definition of T cell receptor signaling thresholds in developing and peripheral mouse T lymphocytes. The activation of SHP-1 is achieved through the engagement of its tandem SH2 domains by tyrosine-phosphorylated proteins; however, the identity of the activating ligand(s) for SHP-1, within mouse primary T cells, is presently unresolved. The identification of SHP-1 ligand(s) in primary T cells would provide crucial insight into the molecular mechanisms by which SHP-1 contributes to in vivo thresholds for T cell activation. Here we present a combination of biochemical and yeast genetic analyses indicating CD22 to be a T cell ligand for the SHP-1 SH2 domains. Based on these observations we have confirmed that CD22 is indeed expressed on mouse primary T cells and capable of associating with SHP-1. Significantly, CD22-deficient T cells demonstrate enhanced proliferation in response to anti-CD3 or allogeneic stimulation. Furthermore, the co-engagement of CD3 and CD22 results in a raising of TCR signaling thresholds hence demonstrating a previously unsuspected functional role for CD22 in primary T cells.

SHP-1,1 an intracellular protein-tyrosine phosphatase, has been demonstrated to be a negative regulator of TCR signaling thresholds (1). SHP-1 is normally maintained in a catalytically inactive state whereby activation minimally requires the engagement of the amino-terminal SH2 domain of SHP-1 by phosphotyrosine (PY)-containing ligand (2, 3). It is predicted that SHP-1-activating ligand(s) exists on mouse naive T cells based on substantial functional evidence indicating SHP-1 to be catalytically active in naive T cells (1, 4–8). It is currently assumed that SHP-1 is activated by one or more components of the TCR signaling pathway. Indeed, the intracellular protein-tyrosine kinase, ZAP-70, has been proposed to bind SHP-1 (9).

However, the best evidence of SHP-1-associating molecules in other hematopoietic cells relates to the family of immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptors (10). In particular, ITIM receptors Ly49 and CD66a associate with SHP-1 in subpopulations of primary T cells, but to date there has been no definition of the ITIM receptors that activate SHP-1 in the majority of mouse primary T cells (11).

In the first instance, we have exploited SHP-1-deficient moth-eaten T cells to assist in the definition of genuine associations between SHP-1 and TCR signaling components in CD3/TCR-stimulated mouse primary T cells. Our results reveal no binding of the CD3 invariant chains or ZAP-70 to SHP-1 in mouse primary T cells following TCR/CD3 ligation. However, by employing pervanadate (PV) to induce a robust tyrosine phosphorylation of cellular proteins in primary T cells, we demonstrated that a glycosylated tyrosyl phosphoprotein of 150 kDa, (pp150) associates with SHP-1 in mouse peripheral T cells. We have identified pp150 as CD22 and confirmed CD22 to be expressed in primary T cells. The expression of CD22 has until now been thought to be restricted to the B cell lineage. However, consistent with CD22 expression in T cells, the co-engagement of CD22 and CD3 results in a raising of TCR signaling thresholds. Remarkably, the absence of CD22 in T cells results in increased responses to CD3 or allogeneic stimulation. These combined findings highlight a previously unrecognized inhibitory role for CD22 in T cells.

**EXPERIMENTAL PROCEDURES**

Cells—C57BL/6J mice heterozygous at the moth-eaten locus were originally obtained from Dr. Leonard Shultz at The Jackson Laboratory (Bar Harbor, ME) and bred under pathogen-free conditions as a source of motheaten mutants or motheaten controls. motheaten mutants or littermate motheaten controls bearing the F5 TCR were as described in Ref. 8. motheaten mutants or littermate motheaten controls were sacrificed between 9–13 days postpartum. CD22 deficient mice (CD22−/−) backcrossed for 10 generations onto the C57/BL6J genetic background were kindly provided by Profes-
sor Michael Neuberger, MRC Laboratory of Molecular Biology, Cambridge, UK. Lymph nodes from 8–12-week old CD22/H11002/ and age-matched control mice were harvested as a source of T cells for proliferation assays. Spleens from 8–12-week old BALB/c mice provided a source of allogeneic feeder cells. For all other experiments, thymi and spleens from 4–12-week old mice kept under pathogen-free conditions were harvested, and cells were isolated as described in Ref. 8. All animal experimentation was in accordance with the UK Animal (Scientific Procedures) Act 1986 under Project Licenses PPL 40/2046 and 30/2125. Dr. Robert L. Geahlen in the Department of Medical Chemistry and Pharmacology at Purdue University kindly provided the mouse T cell lymphoma line, LSTRA, and Professor Elisabeth Simpson at Imperial College generously provided the Abelson virus-transformed B cells (12).

**Generation of T Lymphoblasts**—T lymphoblasts were generated as described in (13). Briefly, mouse splenocytes and thymocytes were stimulated by culturing with 2 μg/ml of concanavalin A for 72 h at 37 °C in complete RPMI 1640 medium supplemented with 360 IU/ml of rIL-2 (Chiron, Harefield, UK). The cells were washed thoroughly and cultured for a further 48 h in complete RPMI 1640 medium supplemented with IL-2. Fluorescence-activated cell sorter analysis performed on the cultured T lymphoblasts revealed no B cell contamination.

**B Cell Purification**—B cells were purified from the spleens of C57Bl6/J and BALB/c mice by positive selection with CD45R Miltenyi microbeads according to the manufacturer's instructions (Miltenyi Biotec, Bisley, Surrey, UK). Purified B cells were lysed in Nonidet P-40 lysis buffer and normalized for protein concentration. Equal volumes of lysate were mixed with 6 μg/mL Laemmli buffer and electrophoresed on a 10% acrylamide SDS-polyacrylamide gel.

**T Cell Stimulation and Lysis**—A total of 5–10 x 10^7 T cells were stimulated for 2 min at 37 °C with 10 μg/ml of hamster anti-CD3 mAb, 2C-11, and rabbit anti-hamster polyclonal serum (Sigma). 2C-11 was kindly provided by Dr. Doreen Cantrell, University of Dundee, Dundee, UK. YO1 cells (14) previously pulsed with either 10 μM agonist (NP-68) or control (GAG) peptide were used as described in Ref. 8. Alternatively, cells were stimulated with 200 μM pervanadate for 10min at 37 °C. Following stimulation, the cells were lysed as described in Ref. 13. Membrane fractions were prepared as described in Ref. 13.

**Immunoprecipitation, Deglycosylation, and Immunoblotting**—SHP-1 FIG. 1. ZAP-70 is not a SHP-1 binding ligand. A, T cell blasts derived from moth-eaten (me/me) and control (me/+ ) thymocytes were left unactivated or activated with anti-CD3 for 5 min, lysed, and subjected to immunoprecipitation (IP) with either anti-SHP-1 serum or anti-CD3 mAb. Immune complexes were resolved by SDS-PAGE and probed for phosphotyrosine (PY)-containing proteins. Blots were then stripped and reprobed for ZAP-70 and SHP-1. B, primary thymocytes from moth-eaten and control mice bearing the transgenic TCR, F5, were activated with YO1 cells pulsed with cognate (NP68) or control (GAG) peptide. Cells were lysed and subjected to immunoprecipitation with either anti-SHP-1 serum or anti-CD3 mAb. Immune complexes were resolved by SDS-PAGE and probed for PY-containing proteins. Blots were then stripped and reprobed for ZAP-70.

**Fig. 2.** pp150 is the major tyrosine-phosphorylated protein associating with SHP-1 in primary T cells. T lymphoblasts from C57BL/6J, CBA, and NOD mice were stimulated with PV, lysed, and subjected to immunoprecipitation (IP) with either pre-immune or anti-SHP-1 sera. Immune complexes were resolved on SDS-PAGE and immunoblotted for PY. The blot was subsequently stripped and reprobed for SHP-1.
was immunoprecipitated using a rabbit polyclonal anti-SHP-1 antibody as described in Ref. 15 or the C-19 antibody (Santa Cruz Biotechnology). CD22 was immunoprecipitated using polyclonal anti-CD22 antisera kindly provided by Dr. Paul Crocker, University of Dundee, Dundee, UK or with a purified rabbit antibody described in Ref. 16. Goat anti-serum to CD3 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Deglycosylation of SHP-1 immunoprecipitates was performed as described in Ref. 13. The immunoprecipitations were resolved by 12% SDS-PAGE, and membranes were probed with anti-PY antibody, 4G10 (Upstate Biotechnology, Lake Placid, NY), and detected by ECL (Amersham Biosciences). The mAb used for immunoblotting ZAP-70 was purchased from BD Transduction Laboratories (Franklin Lakes, NJ). CD22 was immunoblotted with a rabbit antibody raised against exon 12 of mouse CD22 (16).

**Yeast Trihybrid Screen**—Yeast strain YlcK/BDSHP1SH2 was generated as described in Ref. 13, and all yeast manipulations were performed according to standard yeast protocols. Competent yeast cells were prepared and transformed using the lithium acetate/Tris-EDTA/polyethylene glycol protocol. The cDNA for the library was generated from poly(A) RNA extracted from LSTRA lymphoma cells and synthesized using an oligo(dT) primer. cDNA was unidirectionally cloned between the EcoRI and XhoI sites of the yeast cloning vector HybriZAP-2.1 (Stratagene). cDNA library screening was performed using YlcK/BDSHP1SH2 (13). Approximately $3 \times 10^9$ cells were transformed with 100 μg of LSTRA library DNA and plated on minimal agar containing yeast nitrogen base, 2% glucose, and 5 μl 3-amino-1,2,4-triazole, a competitive inhibitor of the HIS3 gene, one of the reporter genes in the YlcK/BDSHP1SH2 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 histidine but supplemented with methionine. Colonies were left to grow for 3 days and then filter-lifted and screened for protein-protein interactions using the lacZ reporter. β-Galactosidase activity was monitored using a freeze-thaw fracture assay. Positive clones were deemed to be those yeast that only turned blue on media lacking methionine. Library plasmid DNA was recovered from yeast by enzymatic disruption of the cell wall by treatment with Zymolyase®-100T (ICN Biomedicals, Costa Mesa, CA), alkaline lysis extraction, and amplification in Max-Efficiency DH5α™ Escherichia Coli (Invitrogen). Three colonies from each positive hit from the yeast β-galactosidase filter assay were grown for 3 days at 30 °C before DNA was extracted using the QIAprep spin miniprep kit (Qiagen). Plasmids were re-introduced into the screening strain or YlcK with the Gal4 binding domain alone to confirm specificity. Sequence data were generated on a 3100 genetic analyzer using ABI Big Dye automated sequencing protocols (PE Applied Biosystems, Foster City, CA).

**Protein Purification and Identification**—A GST fusion protein con-
C57BL/6J mice were stimulated in vitro (20). The acquired tandem mass spectrometry data were analyzed with the non-redundant mouse data base from NCBI using SEQUEST database search tool for peptide identification (21). For the purpose of identifying proteins that bind to SHP-1 in primary T cells, we performed yeast two-hybrid screens (22). We used the C57BL/6J strain as the wild-type mouse strain and the SHP-1-deficient strain as the mutant mouse strain. The yeast strain 2-hybrid system was used to screen for SHP-1-interacting proteins. The yeast strain was transformed with a LSTRA cDNA library prepared from the mouse thymus. After selection of positive clones, we confirmed the interaction with the CD3/TCR triggering of mouse primary T cells. The interaction of CD3/TCR with SHP-1 was detected by co-immunoprecipitation. The results indicated that SHP-1 is one of the SH2 domain-containing proteins that bind to CD3/TCR in mouse primary T cells. Shown in Figure 1 are the results of co-immunoprecipitation of SHP-1 with CD3 or ZAP-70 proteins. In Figure 1A, the major band of 120 kDa was identified as SHP-1. In Figure 1B, the major band of 110 kDa was identified as ZAP-70. In control T cells, no co-immunoprecipitation was detected, indicating that the CD3/TCR triggering of mouse primary T cells does not induce the specific association of SHP-1 with CD3 or ZAP-70 proteins.

**RESULTS**

**ZAP-70 Is Not a Genuine SHP-1 Binding Ligand**—The functional analyses of T cells from SHP-1-deficient moth-eaten mice and T cells expressing a catalytically inactive dominant negative isoform of SHP-1 have demonstrated a role for SHP-1 in contributing to the thresholds of TCR activation (1, 4–9). A corollary to these findings is that SHP-1 must be catalytically active in normal T cells as a consequence of a PY-dependent ligand engagement of its amino-terminal SH-2 domain. To reveal phosphotyrosine-containing proteins that bind to SHP-1 following TCR ligation we performed immunoprecipitations of SHP-1 from primary T cells isolated from SHP-1-deficient and littermate control T cells (Fig. 1A). In control T cells, a PY-containing protein of 72 kDa, established to be ZAP-70 by co-immunoprecipitation with SHP-1 following CD3 triggering, was found co-immunoprecipitated with SHP-1 following CD3 triggering. However, ZAP-70 was also found co-immunoprecipitated with SHP-1 in lysates derived from SHP-1-deficient T cells perhaps because of the inadvertent immunoprecipitation of the activating anti-CD3 antibody, which itself co-immunoprecipitates ZAP-70 (21). To circumvent spurious co-precipitation, SHP-1-deficient and control thymocytes bearing a transgenic TCR, F5, were stimulated with a cognate peptide presented by YO1 cells (Fig. 1B). SHP-1 immunoprecipitations from peptide/antigen-presenting cell-stimulated thymocytes resulted in no co-immunoprecipitation of ZAP-70, although the tyrosine phosphorylation of ZAP-70 could be readily detected in a parallel anti-CD3 co-immunoprecipitation following peptide/antigen-presenting cell stimulation. We therefore concluded that the CD3/TCR triggering of mouse primary T cells does not induce the specific association of SHP-1 with CD3 or ZAP-70 proteins.

**pp150 Is the Major Tyrosine-phosphorylated Protein Associating with SHP-1 in Mouse Primary T Cells**—A difficulty in identifying the molecules associated with SHP-1 in primary T cells is that the physiological stimuli capable of inducing tyrosine phosphorylation of putative SHP-1 ligands are unknown presently. However, the degree of tyrosine phosphorylation on a given protein is an outcome of the counteractive effects of protein-tyrosine kinases and protein-tyrosine phosphatases (22). Consequently, inhibition of protein-tyrosine phosphatases by the potent inhibitor, pervanadate (PV), can lead to an accumulation of PY on those proteins, which are the normal substrates of protein-tyrosine kinases (23). Hence, PV may be utilized to ascertain those molecules potentially capable of associating with SHP-1 under physiological conditions in primary T cells. SHP-1 immunoprecipitations were performed on PV-stimulated splenic T cell blasts derived from a number of mouse strains including C57BL/6J, CBA, NOD, BALB/c, A/J, C3H, SWR, NIH, DBA, FVP, and SJL. The T cell blasts were confirmed by fluorescence-activated cell sorter analysis to have no cell contamination (data not shown). The results demonstrated that for the majority of strains the most prominent PY protein consistently associating with SHP-1 in mouse peripheral T cells is one of 150 kDa (pp150), although additional weak PY proteins of 75 and 45 kDa also were occasionally co-immunoprecipitated (Fig. 2 and results not shown). In contrast to all other strains examined, strikingly, T cells from the strain C57BL/6J showed a much reduced level of tyrosine-phosphorylated pp150 associated with SHP-1 despite an equivalent immunoprecipitation of SHP-1 (Fig. 2 and results not shown). The differential association of pp150 and SHP-1 in T cells from C57BL/6J mice may be because of one or more genetic differences in the expression of the pp150 receptor, its ability to be tyrosine-phosphorylated and to associate with SHP-1, or a combination of these possibilities. Furthermore, anti-CD3 stimulation of T cells from the mouse strain BALB/c resulted in no pp150 association with SHP-1, although pp150 was readily detected in association with SHP-1 following PV treatment of the same T cells (Fig. 3).

**pp150 Is an N-Glycosylated, Membrane-associated Protein**—We examined whether pp150 might represent a membrane-associated receptor. As the possession of N-linked carbohydrates is a common feature of plasma membrane receptors, SHP-1 was immunoprecipitated from a lysate of T cells of the CBA strain, and the immunoprecipitate was subjected to Endo F treatment. pp150 was indeed found to be N-glycosylated, as Endo F treatment accelerated its migration on SDS-PAGE to ~130 kDa (Fig. 4A). We also performed SHP-1 immunoprecipitations on the P100 (membrane) fraction of PV-stimulated T cells and confirmed that pp150 is a membrane-associated protein (Fig. 4B). These results indicate that pp150 is a cell surface transmembrane receptor with an N-glycosylated extracellular domain.

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**Table 1**

| Clone | Encoded protein | Residue numbers | No. of isolates |
|-------|-----------------|-----------------|----------------|
| A1–4  | gp49B1          | 235–280         | 4              |
| A5    | CD72            | 1–199           | 1              |
| A6    | CD22            | 767–868         | 1              |

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**Note:** All clones were isolated from LSTRA cDNA libraries prepared from normal mouse thymus.

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pp150 Is Identified as CD22—Previously, we reported a tyrosyl phosphoprotein around 150–160 kDa associated with SHP-1 in the mouse lymphoma, LSTRA, and it is possible that this protein corresponds to pp150 in primary T cells (17). To identify pp150, a cDNA library was generated in the yeast-cloning vector, HybriZAP-2.1 using mRNA from the LSTRA cells. The HybriZAP-2.1 vector directs the expression of hybrid proteins encompassing the yeast Gal4 activation domain. The cDNA library was used to transform a stable yeast strain, Ylck/BDSHP1SH2 (13), that expresses 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) of SHP-1 fused to the DNA binding domain of Gal4. In this yeast trihybrid vector system, the regulatable expression of Lck permits the identification of interactions between the SH2 domains of SHP-1 and tyrosine-phosphorylated ligands (13). The transformation of Ylck/BDSHP1SH2 with the LSTRA cDNA library resulted in the identification of partial cDNAs encoding for the ITIM-containing receptors gp49B1, CD72, and CD22 (Table I). All cDNAs encoded for the ITIM-containing cytoplasmic region of each receptor. Of the three proteins, only the known molecular mass of CD22 (150 kDa) (24), as opposed to gp49B1 (49 kDa) (25) and CD72 (45 kDa) (26), is consistent with the size of the major tyrosyl phosphoprotein (pp150) found co-immunoprecipitating with SHP-1 in primary T cells. However, thus far, only CD72 has been reported to be expressed on mouse primary T cells (27).

To confirm the identity of the LSTRA-derived pp150 protein, we purified this protein by affinity chromatography using a GST fusion protein column containing the SH2 domains of SHP-1 (17). LSTRA cell lysates were incubated with purified GST-Sh2/SHP-1 fusion protein, purified anti-CD22 antibodies (CD22), or pre-immune serum (PI). Immune complexes were resolved by SDS-PAGE and immunoblotted for PY. As a control for protein loading, whole cell lysates (WCL) were loaded in parallel. D, LSTRA cells were subjected to immunoprecipitation with either pre-immune, anti-SHP-1, or anti-CD22 sera. Immune complexes were resolved on SDS-PAGE and immunoblotted for CD22 and SHP-1. As a control for protein loading, whole cell lysates were electrophoresed in parallel and immunoblotted for CD22 and SHP-1.

**FIG. 5. Identification of pp150 in LSTRA.** A, LSTRA cell lysates were incubated with purified GST-Sh2/SHP-1 fusion protein or GST alone. The bound proteins were deglycosylated, resolved by SDS-PAGE, and subjected to immunoblot analysis with anti-phosphotyrosine antibodies (left panel) or visualized by silver staining (right panel). B, the bands corresponding to 150 and 110 kDa were subjected to mass spectrometric analysis. The upper panel shows peptide sequences of CD22 identified by tandem mass spectrometric analysis; the lower panel demonstrates a tandem mass spectrum of a peptide derived from collision-induced dissociation of the (M+2H)2+ precursor, m/z 950.3. This peptide was identified as common to both the 110- and the 150-kDa bands. C, LSTRA cell lysates were incubated with purified GST-Sh2/SHP-1 fusion protein, purified anti-CD22 antibodies (CD22), or pre-immune serum (PI). Immune complexes were resolved by SDS-PAGE and immunoblotted for PY. As a control for protein loading, whole cell lysates (WCL) were loaded in parallel. D, LSTRA cells were subjected to immunoprecipitation with either pre-immune, anti-SHP-1, or anti-CD22 sera. Immune complexes were resolved on SDS-PAGE and immunoblotted for CD22 and SHP-1. As a control for protein loading, whole cell lysates were electrophoresed in parallel and immunoblotted for CD22 and SHP-1.
bands as CD22 (Fig. 5B), and immunoblot analysis using anti-CD22 antibodies confirmed this finding (Fig. 5C). Because CD22 is tyrosine-phosphorylated constitutively in LSTRA, one might expect SHP-1 to be bound constitutively to CD22 in LSTRA cells. Indeed, this can be readily demonstrated in reciprocal CD22 and SHP-1 immunoprecipitations from untreated LSTRA cells (Fig. 5D).

To investigate whether the pp150 detected associating with SHP-1 on primary T cells corresponds to CD22, SHP-1 immunoprecipitations from T lymphoblasts generated from BALB/c and C57BL/6J strains were immunoblotted for CD22. Fig. 6A demonstrates that CD22 is indeed identical to SHP-1-associated pp150. Interestingly, the amount of CD22 associating with SHP-1 is significantly reduced in C57BL/6J- versus BALB/c-derived T cells. This result provides an explanation for the previous finding that the amount of tyrosine-phosphorylated pp150 associating with SHP-1 is reduced in C57BL/6J-derived T cells. The strain differences may reflect a reduction in the T cell expression of CD22 or an inability of CD22 to associate with SHP-1 in T cells of the C57BL/6J strain. To distinguish between these possibilities, CD22 was directly immunoprecipitated from T lymphoblasts generated from BALB/c and C57BL/6J strains and immunoblotted for CD22. Fig. 6B indicates that the level of expression of CD22 is reduced in T cells derived from the C57BL/6J strain. However, the strain differences in CD22 expression are restricted to T lymphocytes, because the level of expression of CD22 in B lymphocytes is equivalent between the two strains (Fig. 6C).

Ligation of CD22 on Naive T Cells Inhibits Anti-CD3-induced Proliferation—By employing a secondary amplification step during flow cytometry analysis, a low level expression of CD22 over background was detected on T cells of both BALB/c- and C57BL/6J-derived naive T cells (Fig. 7). As a control, no CD22 expression was detected on the surface of CD22−/− T cells (Fig. 7). Given the proven role of CD22 as a negative regulator of BCR signaling thresholds in B cells (28–31), it is conceivable that CD22 expressed on T cells would have an equivalent role in regulating TCR signals. Splenic T cells from either BALB/c or C57BL/6J mice were stimulated in vitro with a titration of plate-bound anti-CD3 in conjunction with either anti-CD22 or an isotype control antibody. Fig. 8A demonstrates that the co-engagement of CD22 with CD3 on BALB/c T cells resulted in a significant reduction in T cell proliferation. Co-engagement of CD22 with CD3 on C57BL/6J T cells also produced inhibition, albeit to a lesser degree (Fig. 8B).

Absence of CD22 on Naive T Cells Enhances Anti-CD3-induced Proliferation—As further proof of the physiological relevance of CD22 in naive T cells we examined the proliferation of T cells isolated from CD22-deficient versus age-matched control C57BL/6J mice. T cells were stimulated by plate-bound anti-CD3 in conjunction with anti-CD22. Anti-CD22 was included in the assay based on the previous observation that addition of anti-CD22 can inhibit anti-CD3-induced proliferation (Fig. 8A). This method of T cell stimulation was expected to enhance any proliferative differences between CD22-deficient and control T cells. Fig. 9A demonstrates that CD22-deficient T cells indeed have an increased sensitivity to triggering by plate-bound anti-CD3. In addition, CD22-deficient cells in comparison to control T cells, demonstrated exaggerated proliferative responses when stimulated with the indicated ratios of allogeneic BALB/c stimulator cells (Fig. 9B). Taken together, these results highlight an important and unappreciated physiological role for CD22 in influencing TCR/CD3 complex signaling thresholds.

DISCUSSION

The requirement for tandem engagement of the SH2 domains of SHP-1 (3) dictates that the most efficient activating ligands for SHP-1 are molecules possessing a minimum of two ITIMs. The observation of a tyrosine-phosphorylated, membrane-associated, glycosylated protein binding to SHP-1 in T cells led us to hypothesize that this would be an ITIM-bearing molecule. Consequently, a genetic screen of a cDNA library generated from the mouse lymphoma, LSTRA, was performed that resulted in the identification of the tandem ITIM-bearing receptor CD22 as a candidate for pp150. This was a surprising result given that CD22 has been previously characterized as a B cell-restricted antigen (24). Nevertheless, mass spectrometric analysis conducted in parallel confirmed pp150 as CD22 in LSTRA cells. Furthermore, by direct immunoprecipitation and immunoblotting, we were able to demonstrate the presence of CD22 in primary T cells. A level of CD22 was also detectable by
flow cytometry on the surface of primary T cells. Furthermore, it is clear that the low level of CD22 on primary T cells is sufficient to confer the functional effects on TCR-induced proliferation. Conceivably, CD22 may be involved in additional regulatory roles in T cells.

In addition to CD22, the yeast trihybrid screen also identified the ITIM receptors gp49 and CD72 as interacting with SHP-1. CD72 has been previously demonstrated to be expressed on a fraction of mouse peripheral T cells (27) and may correspond to the pp45 detected associating with SHP-1 in Fig. 2. This current study has focused on pp150, and hence no direct immunoblotting has been performed for gp49 and CD72.

Differences in the ability of T cells to undergo proliferation (32, 33) or in alterations in T helper cell polarization (34, 35) have been reported between mice from distinct genetic backgrounds. We hypothesize that the differential expression of CD22 in T cells from the C57BL/6J versus other strains may result in distinct alterations in T cell biology. Indeed, it is apparent that the engagement of CD22 in T cells of the BALB/c strain has a more marked inhibitory effect on TCR-triggered proliferation in comparison to the C57BL/6J strain. Possibly because of the low level of CD22 signal detected by flow cytometry on T cells, the differential expression of CD22 in C57BL/6J versus BALB/c T cells is more readily apparent by immunoblotting (especially PY immunoblotting following SHP-1 immunoprecipitation). A direct sequel of our observations is that CD22-deficient T cells would also be predicted to hyperproliferate in response to TCR stimulation. Although all CD22-deficient mice have thus far been generated in the C57BL/6J genetic background (28–31), wherein differences in T cell behavior are less likely to be found, the detectable expression of CD22 in C57BL/6J T cells could nevertheless be sufficient to produce functional effects. Indeed, an examination of CD22-deficient T cells on the C57BL/6J genetic background confirmed a role for
whether a CD22-SHP-1 complex is likely to be generated as a direct negative feedback of TCR signaling. However, we presume a level of tyrosine phosphorylation of CD22 below detection in our assays but sufficient for recruitment of SHP-1 must be occurring in either unstimulated and/or CD3-stimulated naïve T cells to account for our functional data with the anti-CD22 antibody and CD22-deficient T cells. Furthermore, the functional effects achieved here by simultaneous engagement of CD22 and CD3 by antibodies hint at a possible important role played by CD22 ligands in positioning a CD22-SHP-1 complex in proximity to the TCR. In addition, it remains possible that a more extensive phosphorylation of CD22 can occur in T cells in response to physiological stimuli other than TCR engagement. Likewise, it is conceivable that similar stimuli may also influence the tyrosine phosphorylation state of CD72 expressed on T cells. Under such conditions, CD72 could recruit SHP-1 and thereby modulate T cell activation. The elucidation of these tyrosine phosphorylation-inducing stimuli will provide insight into how T cells integrate signals from accessory receptors like CD22 and CD72 into the establishment of TCR signaling thresholds.

We have previously demonstrated that SHP-1 associates with the ITIM receptor, LAIR-1, in Jurkat and primary human T cells (13). Although a mouse homologue of LAIR-1 has been identified, our biochemical and yeast genetic analyses have not revealed mLAIR-1 to be a SHP-1 ligand in the context of T cells. The lack of association of SHP-1 and mLAIR-1 may possibly be attributed to the imperfect second ITIM in the cytoplasmic domain of mLAIR-1 (36). In summary, it is likely that T cells use multiple ITIM receptors like CD22, CD72, and LAIR-1 to reset their TCR thresholds in response to different extracellular cues.

In conclusion, we report a SHP-1-recruiting membrane-associated molecule, pp150, in mouse peripheral T cells and identify pp150 as CD22. Engaging CD22 inhibits T cell proliferation in response to TCR triggering, and CD22 deficiency confers a hyperproliferative phenotype to primary T cells. We believe that these findings will focus attention on CD22 as an important regulator of T cell function in addition to its established role in B cells.

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CD22 in inhibiting TCR-induced proliferation.

It is evident that the expression of CD22 is significantly lower in T versus B cells. This is likely to be attributed to cell-specific regulatory proteins that govern CD22 expression. The molecular basis for the lower expression of CD22 in T cells of the C57BL/6J strain remains to be established but this difference clearly does not extend to B lymphocytes of the same strain. Gene mapping studies are in progress to establish whether the reduced expression of CD22 in T cells of the C57BL/6J strain can be directly attributed to polymorphisms within the cd22 gene.

It is apparent from the functional data presented here that CD22 can exert an effect on T cell activation mediated through the TCR. However, our biochemical analyses indicated that TCR signaling does not cause induction of detectable levels of CD22 tyrosine phosphorylation. It is therefore uncertain whether CD22-deficient T cells hyperproliferate upon TCR triggering. Purified lymph node T cells from CD22−/− and age-matched C57BL/6/J mice were dispensed into 96-well plates and stimulated for 72 h with either a titration of immobilized anti-CD3 antibody (A) or with the indicated ratios of irradiated allogeneic BALB/c stimulator cells (B). T cell proliferation was assessed by the incorporation of [3H]thymidine during the final 16 h of culture. Asterisks indicate a significant difference at the $p \leq 0.05$ level of significance.

FIG. 9. CD22-deficient T cells hyperproliferate upon TCR triggering. Purified lymph node T cells from CD22−/− and age-matched C57BL/6/J mice were dispensed into 96-well plates and stimulated for 72 h with either a titration of immobilized anti-CD3 antibody in conjunction with 5 μg/ml of anti-CD22 antibody (A) or with the indicated ratios of irradiated allogeneic BALB/c stimulator cells (B). T cell proliferation was assessed by the incorporation of [3H]thymidine during the final 16 h of culture. Asterisks indicate a significant difference at the $p \leq 0.05$ level of significance.

CD22 is lower in T cells of the C57BL/6J strain. Whether the reduced expression of CD22 in T cells of the C57BL/6J strain can be directly attributed to polymorphisms within the cd22 gene.
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**CD22 in Primary T Cells**

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CD22 Is a Functional Ligand for SH2 Domain-containing Protein-tyrosine Phosphatase-1 in Primary T Cells
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