Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8959–8963, November 1989
Immunology

Expression of CD45 alters phosphorylation of the *lck*-encoded tyrosine protein kinase in murine lymphoma T-cell lines

(L-CA/T200/p56^{lck}/phosphotyrosine phosphatase/lymphocyte growth regulation)

HANNE L. OSTERGAARD*, DEBORAH A. SHACKELFORD*, TAMARA R. HURLEY†, PAULINE JOHNSON*, ROBERT HYMAN*, BARTHOLOMEW M. SEFTON†, AND IAN S. TROWBRIDGE*

*Department of Cancer Biology and †Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138

Communicated by Robert W. Holley, August 1, 1989 (received for review, June 27, 1989)

ABSTRACT **CD45 is a family of high molecular weight leukocyte cell surface glycoproteins. Recently, two related subregions of the cytoplasmic domain of CD45 have been shown to have 30–40% amino acid identity with a human placental protein phosphotyrosine phosphatase, and CD45 isolated from human spleen was found to exhibit intrinsic protein phosphotyrosine phosphatase (EC 3.1.3.48) activity. In the present studies, we demonstrate that each of the known isoforms of murine CD45 has an equivalent basal level of protein phosphotyrosine phosphatase activity and establish that this enzymatic activity is associated with the cytoplasmic domain of the glycoprotein. Studies with three independent sets of well-characterized parental CD45$^+$, mutant CD45$^-$, and revertant CD45$^+$ lymphoma cell lines indicate that loss of CD45 increases the phosphorylation of the *src*-related leukocyte-specific tyrosine protein kinase p56^{lck} on tyrosine-505, a putative negative regulatory site. This suggests that CD45 may play a role in leukocyte growth regulation by altering the kinase activity of p56^{lck}.**

CD45 (T200 or L-CA) is a family of major leukocyte-specific cell surface glycoproteins expressed exclusively on hematopoietic cells (reviewed in ref. 1). Isoforms of CD45 are generated by the alternative splicing of three exons, each encoding ≈50 amino acids that are inserted near the amino terminus of the molecule. Of the eight possible mRNAs that can be generated by the differential use of the three exons, six have been identified by sequencing CD45 cDNAs from mouse, human, and rat (2–6). The extra sequences of CD45 encoded by the alternatively spliced exons contain multiple sites for O-linked oligosaccharides, and at least one extra segment is known to be extensively glycosylated (7). Different isoforms of CD45 are selectively expressed on specific subpopulations of hematopoietic cells, and changes in the pattern of expression of CD45 isoforms in T cells also occur upon antigenic stimulation (8–10). CD45 is also distinguished by a large, highly conserved, cytoplasmic domain of 705 amino acids that can be subdivided into two related subdomains of ≈300 amino acids. Recently, each of these subdomains was shown to have 30–40% amino acid identity with a soluble human placental protein phosphotyrosine phosphatase (PTPase; protein-tyrosine phosphatase, EC 3.1.3.48), and CD45 isolated from human spleen was found to have intrinsic PTPase activity (11, 12). We have extended these observations by analyzing the PTPase activity of individual isoforms of murine CD45 and truncated forms of the molecule. Further, the availability of three independent sets of parental CD45$^+$, mutant CD45$^-$, and revertant CD45$^+$ lymphoma cells has allowed us to search for *in vivo* substrates of the CD45 PTPase activity by comparing the phosphotyrosine-containing proteins in CD45$^+$ and CD45$^-$ cells. The results of this analysis indicate that loss of CD45 in the mutant lymphoma cells correlates with increased phosphorylation of the *src*-related leukocyte-specific tyrosine protein kinase p56^{lck} (13, 14) at Tyr-505, a putative negative regulatory site (15, 16). Previous studies using monoclonal antibodies have implicated CD45 in a variety of lymphocyte functions, including proliferative responses and signal transduction (17–19). Our data suggest that one possible mechanism by which CD45 might play a role in lymphocyte growth regulation is by directly, or indirectly, modifying the kinase activity of p56^{lck}.

MATERIALS AND METHODS

Cell Lines. CD45$^-$ mutant murine lymphoma cell lines were derived from mutagenized parental cell lines by immunoselection with antibody and complement (20, 21). Their relative levels of CD45 expression are shown in Table 1. To obtain ψ-2 cell lines expressing specific isoforms of CD45, PA-12 cells were transiently transfected with pARV-1 retroviral constructs encoding each of four murine CD45 isoforms (22). After 48 hr, the culture supernatants were harvested and used to infect ψ-2 cells. These cells were then selected in G418, and individual colonies were isolated and subsequently analyzed for CD45 expression. All the transfected cell lines expressed comparable amounts of CD45 as determined by flow cytometry and metabolic labeling with [^{35}S]methionine.

Baculovirus Expression of the CD45 Cytoplasmic Domain. Site-directed mutagenesis was used both to generate a new *Xba* I site 16 base pairs upstream of the ATG start site and to delete amino acid residues 4–563 from a full-length murine CD45 cDNA (4) inserted in pBluescript SK (Stratagene). The *Xba* I fragment containing the coding sequence was inserted into the *Bam*HI site of a baculovirus expression vector (23) and cotransfected with wild-type baculovirus DNA into Sf9 cells to generate recombinant virus. Plaque-purified recombinant virus was used to infect Sf9 cells and direct the synthesis and secretion of the 95-kDa cytoplasmic domain of murine CD45.

Phosphatase Assay. Cells were lysed in 1% Nonidet P-40/0.15 M NaCl/0.01 M sodium phosphate buffer (pH 7.2). Immunoprecipitates prepared with monoclonal antibody I3/2 coupled to Sepharose 4B were washed with 0.2% Nonidet P-40 in 0.15 M NaCl/10 mM sodium phosphate buffer (pH 7.2) (24, 25). Immunoprecipitates from 1–4 × 10^5 cells were then incubated with 0.4 nM [Val^5]angiotensin II (Sigma) that had been phosphorylated by p56^{lck} from LSTRA cells to a specific activity of 1 × 10^7 cpm/pmol (26). Reactions (total volume of 5 μl) were performed in 1 mM EDTA/25 mM imidazole·HCl, pH 7.2 (phosphatase buffer) at 30°C and were stopped by boiling. One microliter from each reaction mixture was spotted onto cellulose thin-layer plates and sub-

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

Abbreviation: PTPase, phosphotyrosine phosphatase.

Table 1. Expression of CD45 on murine lymphoma cell lines

Cell line	Relative amount of antigen	
	CD45 (T200)	Thy-1
BW5147	26.2	29.7
BW5147 (T200$^-$a)5.1	1.1*	69.0
BW5147 (Rev)1.1	4.2	66.0
NZB.1	10.8	23.3
NZB.1 (T200$^-$).4†	1.1*	50.0
NZB.1 (Rev).2†	21.6	39.3
SAKRTLS 12.1	8.9	22.8
SAKRTLS 12.1 (T200$^-$a).5†	1.0	38.3

The relative amount of antigen was measured by the mean logarithm of the fluorescence estimated by flow cytometry (21) with background fluorescence set at 1.0 for each cell line.

*BW5147 (T200$^-$a) and NZB.1 (T200$^-$).4 cells synthesize little or no CD45 as judged by metabolic labeling with [^{35}S]methionine (ref. 20; H.L.O., unpublished data).

†These lines were derived by R.H. and V. Stallings (unpublished data).

jected to electrophoresis in buffer at pH 3.5 for 14 min at 1.5 kV (27). The separated angiotensin and $^{32}P_i$ were located by autoradiography (12 hr at −70°C), the areas of the plates containing $^{32}P_i$ and labeled angiotensin were scraped, and the Cherenkov cpm were determined. The results were expressed as the percent of the total radioactivity recovered in $^{32}P_i$.

Immunoblots. Cells were lysed at 2–4 × 10^7 cells per ml in 1% Nonidet P-40/0.15 M NaCl/2.5 mM EDTA/0.5 mM sodium orthovanadate/10 mM sodium phosphate buffer (pH 7.5) at 4°C, and then immunoprecipitates were prepared as described (28) with rabbit anti-p56^{lck} antiserum (26). The cell lysates (1.2 × 10^6 cell equivalents) and immunoprecipitates (5 × 10^6 cell equivalents) were subjected to electrophoresis on an SDS/8.5% polyacrylamide gel and then transferred to nitrocellulose as described by Towbin *et al.* (29) with a Novex Western transfer apparatus. Immunoblotting was performed with rabbit anti-p56^{lck} antiserum (26) or affinity-purified rabbit anti-phosphotyrosine antibodies (30). Prestained SDS electrophoresis molecular size markers (Sigma) were used as standards.

Phospho Amino Acid Analysis and Peptide Maps. SAKRTLS 12.1 and SAKRTLS 12.1 (T200$^-$) cells (4 × 10^7) were metabolically labeled with 5 mCi of $^{32}P_i$ (ICN; specific activity = 285 Ci/mg; 1 Ci = 37 GBq) for 6 hr and then lysed by boiling in 0.5% SDS/1 mM dithiothreitol/10 mM Tris·HCl (pH 8.0). Lysates were adjusted to RIPA buffer (26), and immunoprecipitates were prepared with a rabbit anti-p56^{lck} serum. The immunoprecipitates were subjected to electrophoresis on an SDS/10% polyacrylamide gel and transferred to Immobilon (Millipore) for phospho amino acid analysis and to nitrocellulose for tryptic digestion. After they were localized by autoradiography, the p56^{lck} bands were excised. For phospho amino acid analysis, the band containing p56^{lck} was treated with 200 μl of 5.7 M HCl for 1 hr at 110°C, dried, and resuspended in 10 μl of H_2O (31). A total of 200 cpm were spotted onto a cellulose thin-layer plate and analyzed by electrophoresis at pH 1.9 in the first dimension and at pH 3.5 in the second dimension (27). For two-dimensional tryptic peptide mapping, p56^{lck} (280 cpm) was digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington) and the resulting peptides were separated by electrophoresis at pH 8.9 in the first dimension toward the positive electrode (left side) and in the second dimension by ascending chromatography as described (27).

RESULTS AND DISCUSSION

PTPase Activity of Murine CD45: Localization to the Cytoplasmic Domain. To determine the intrinsic PTPase activity of murine CD45, the glycoprotein was isolated by immunoprecipitation with monoclonal antibody I3/2 (24) and then assayed for PTPase activity by using ^{32}P-labeled angiotensin as substrate (32). Immunoprecipitates containing CD45 from parental CD45$^+$ cells had more than a 100-fold greater PTPase activity than similar immunoprecipitates from CD45$^-$ cells (Fig. 1*a*) or immunoprecipitates from CD45$^+$ cells prepared with an irrelevant antibody (data not shown). Immunoprecipitates prepared from a revertant cell line, derived from the NZB.1 (T200$^-$) mutant, reproducibly contained higher PTPase activity than those from the parental cell line. This

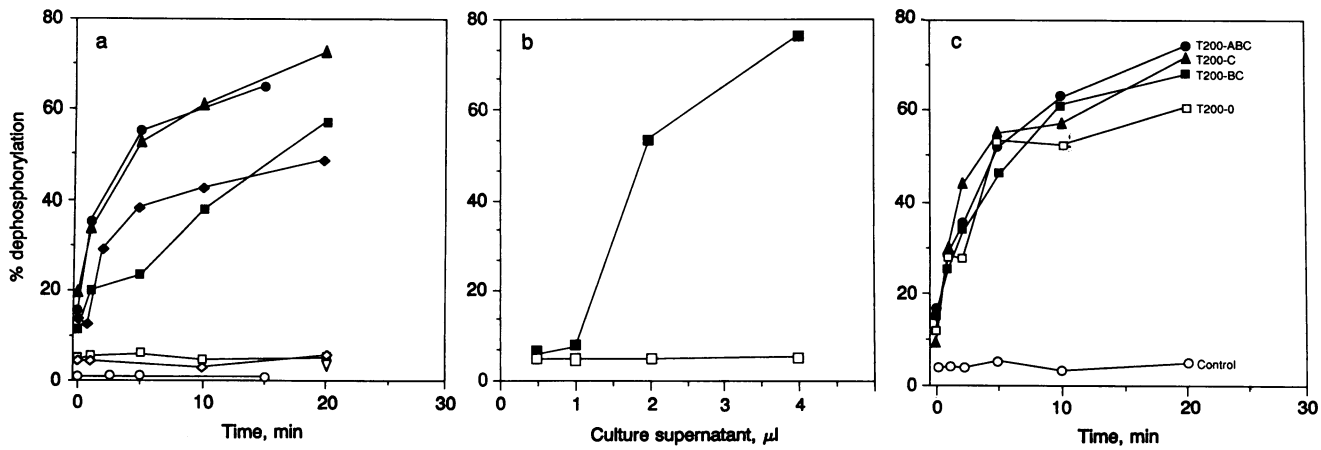

FIG. 1. PTPase activity of CD45. (*a*) PTPase activity of CD45 immunoprecipitates from parental and revertant CD45$^+$ and mutant CD45$^-$ lymphoma cell lines (see Table 1). ◆, BW5147; ▽, BW5147 (T200$^-$a)5.1; ◇, BW5147 (Rev)1.1; ■, NZB.1; □, NZB.1 (T200$^-$).4; ▲, NZB.1 (Rev).2; ●, SAKRTLS 12.1; ○, SAKRTLS 12.1 (T200$^-$a).5. The number of cell equivalents used for each experiment was 2 × 10^5 for the BW5147 cell lines, 3 × 10^5 for the NZB.1 cell lines, and 7.5 × 10^5 for the SAKRTLS 12.1 cell lines. (*b*) PTPase activity of recombinant baculovirus murine CD45 cytoplasmic domain produced in Sf9 insect cells. The PTPase activity of tissue culture supernatant from Sf9 cells infected with either recombinant virus encoding the cytoplasmic domain of murine CD45 (■) or, as a control, cells infected with wild-type virus (□) is shown. Sf9 cells were grown in serum-free medium and supernatants were dialyzed against phosphatase buffer before assay. (*c*) PTPase activity of CD45 immunoprecipitates from ψ-2 cells infected with pARV-1 retroviral constructs expressing isoforms of CD45 containing different combinations of sequences encoded by alternatively spliced exons A, B, and C (22) (T200-0, no alternatively spliced exons; T200-C, exon C only; T200-BC, exons B and C; T200-ABC, exons A, B, and C; control, ψ-2 cells infected with the pARV-1 retroviral vector).

revertant expresses higher levels of CD45 than parental cells (Table 1), and the molecule it expresses is similar in molecular mass to the wild-type glycoprotein.

The 30–40% amino acid identity of the two subdomains of the murine CD45 cytoplasmic domain with a human placental PTPase (11) suggests that the PTPase activity of CD45 is localized to this region of the molecule. To test this directly, we used a previously characterized CD45$^+$ revertant, derived from a CD45$^-$ mutant of BW5147, that expresses, on its cell surface, a truncated form of CD45 glycoprotein lacking most, if not all, of the cytoplasmic domain (21) (Table 1). CD45 from this revertant possessed no detectable PTPase activity (Fig. 1*a*). Furthermore, a recombinant baculovirus protein consisting of only the cytoplasmic domain of murine CD45 expressed in insect cells has PTPase activity *in vitro* (Fig. 1*b*). We do not yet know whether both or only one of the related cytoplasmic subdomains of CD45 are active. Each of the four known isoforms of murine CD45 has been independently expressed in ψ-2 cells by using a retroviral vector (22). As shown in Fig. 1*c*, these isoforms were equally active as PTPases *in vitro*.

Evidence That p56^{lck} Is a Substrate of CD45 *in Vivo*. We investigated the *in vivo* substrates of the CD45 PTPase by immunoblotting total cell protein with anti-phosphotyrosine antibodies (30) (Fig. 2). Three to five major phosphotyrosine-containing proteins that did not differ in their phosphotyrosine content were detected in all three sets of mutant and parental lymphoma cell lines. A major 110-kDa phosphotyrosine-containing protein was prominent in the mutant and revertant NZB.1 cell lines but was not seen in the NZB.1 parental cell line. This difference was not seen in the other sets of cell lines and presumably reflects clonal variation unrelated to the expression of CD45. As the phosphorylation of most of the major phosphotyrosine-containing proteins in lymphoma cells is unaffected by the expression of CD45, either CD45 has a restricted range of substrates or the phosphorylation of these proteins is subject to regulation by other PTPases or phosphotyrosine protein kinases.

One phosphotyrosine-containing protein of approximately 50-kDa, however, was more prominent in all three mutant CD45$^-$ and the BW5147 revertant cell lines (Fig. 2). Thus, the increased phosphotyrosine content of this protein correlated with the loss of CD45. The protein was identified as p56^{lck}, the *src*-related, membrane-associated, cytoplasmic tyrosine protein kinase that is expressed predominantly in T cells (13, 14), by immunoblotting of anti-p56^{lck} precipitates with anti-phosphotyrosine antibodies (Fig. 2). The p56^{lck} isolated from the CD45$^-$ mutant cell lines and the BW5147 revertant lacking CD45 PTPase activity reacted more strongly with anti-phosphotyrosine antibodies than the p56^{lck} from the respective parental cells or the NZB.1 (Rev).2 revertant cells that contain CD45 PTPase activity. These differences in reactivity with anti-phosphotyrosine antibodies reflect increases in the phosphotyrosine content of p56^{lck} rather than increases in the level of the protein, as immunoblotting of the same immunoprecipitates and lysates and antibodies to p56^{lck} showed similar levels of the protein in the cells (Fig. 2).

Increased phosphotyrosine in p56^{lck} from CD45$^-$ cells was also detected by phospho amino acid analysis of p56^{lck} from cells metabolically labeled with $^{32}P_i$ (Fig. 3 *a* and *b*). Whereas p56^{lck} from CD45$^+$ cells contained more phosphoserine than phosphotyrosine, p56^{lck} from CD45$^-$ cells contained more phosphotyrosine than phosphoserine. There is, therefore, an inverse correlation between the level of expression of CD45 and the phosphorylation of p56^{lck} on tyrosine. This suggests that the phosphotyrosine content of p56^{lck} is regulated by

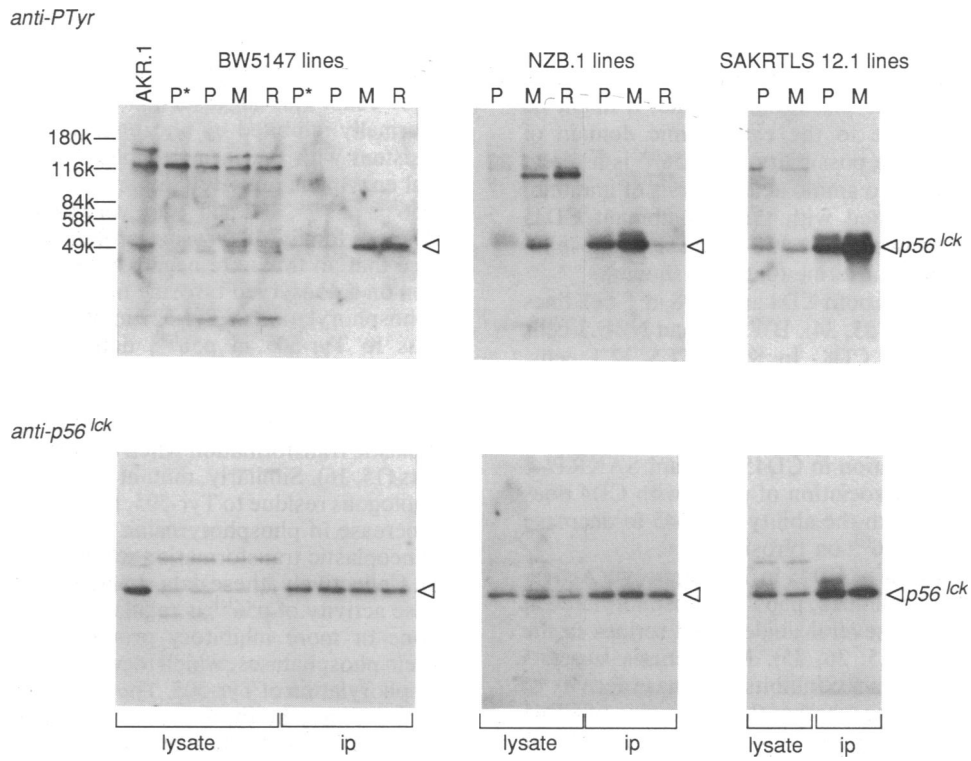

FIG. 2. Immunoblots of cell lysates (lysate) and immunoprecipitates (ip) of p56^{lck} from CD45$^+$ parental (P) and revertant (R) cells and CD45$^-$ mutant (M) cells (see Table 1). (*Upper*) Anti-phosphotyrosine (anti-*P*Tyr) immunoblots. (*Lower*) Anti-p56^{lck} immunoblots. The BW5147 parental cell line (P) differs from the BW5147 cell line (P*) in that it is resistant to tubercidin, deoxygalactose, and diaminopurine. As determined by scanning the autoradiograph with a LKB laser densitometer, the level of phosphorylation of p56^{lck} in NZB.1 (T200$^-$).4 and SAKRTLS 12.1 (T200$^-$) cells was increased 1.5- and 2.5-fold, respectively, relative to p56^{lck} in the corresponding parental cell lines. The level of phosphorylation of p56^{lck} in the NZB.1 (Rev).2 cells was decreased by 60% relative to p56^{lck} in the NZB.1 parental cells correlating with a 2-fold higher level of CD45 on the revertant cells. AKR.1 is a murine T-lymphoma cell line that expresses high levels of p56^{lck}.

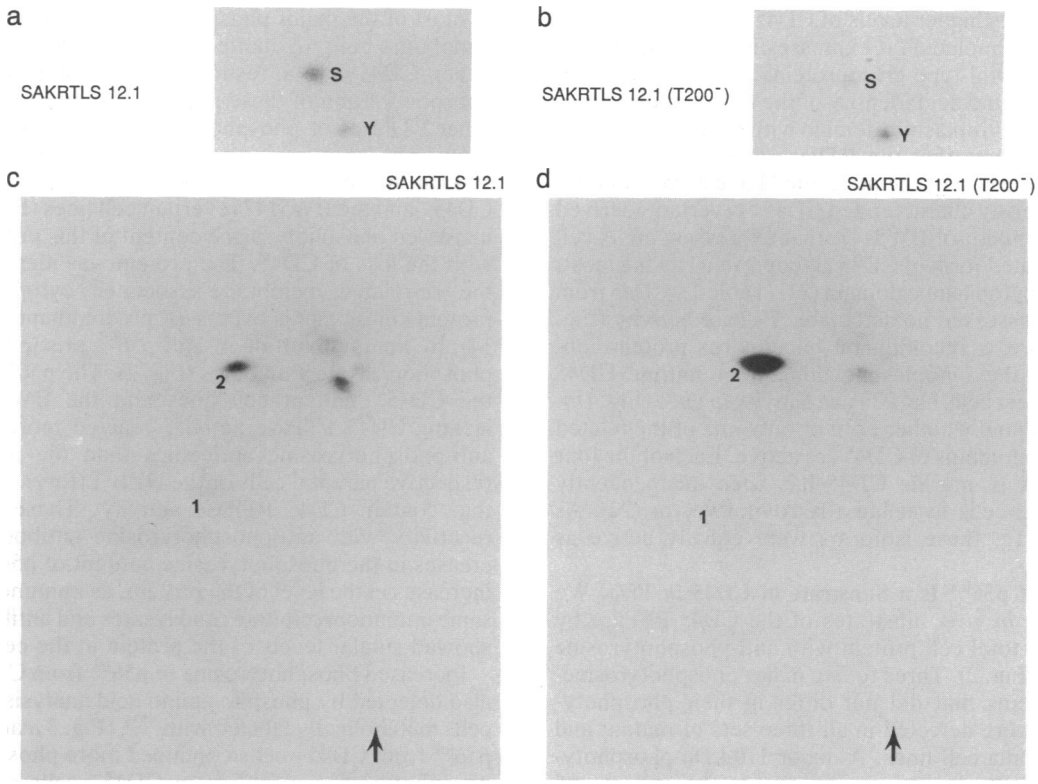

FIG. 3. Phospho amino acid analysis (*a* and *b*) and two-dimensional tryptic peptide mapping (*c* and *d*) of $p56^{lck}$ from parental and mutant SAKRTLS 12.1 cell lines metabolically labeled with $^{32}P_i$. S, phosphoserine; Y, phosphotyrosine. The origin on each peptide map is indicated by an arrow. Peptide 1 contains Tyr-394, and peptide 2 contains Tyr-505.

CD45 *in vivo*. One possibility is that $p56^{lck}$ is a direct substrate of CD45. Alternatively, CD45 could modulate the activity of another PTPase or tyrosine protein kinase that regulates the phosphorylation of $p56^{lck}$ on tyrosine. As $p56^{lck}$ is found at the cytoplasmic face of the plasma membrane, it might be expected to be accessible to the cytoplasmic domain of CD45. Consistent with the possibility that $p56^{lck}$ is a direct substrate of CD45, phospho amino acid analysis of immunoprecipitated $p56^{lck}$ incubated with the recombinant CD45 cytoplasmic domain showed a specific decrease in phosphotyrosine relative to phosphoserine (data not shown).

$p56^{lck}$ is complexed with both CD4 and CD8 in T-cell lines expressing these antigens (33, 34). BW5147 and NZB.1 cells express neither CD4 nor CD8. In SAKRTLS 12.1 cells, however, more than 90% of $p56^{lck}$ was physically associated with CD4 (data not shown). As tyrosine phosphorylation of $p56^{lck}$ in $CD45^+$ parental SAKRTLS 12.1 cells is reduced relative to its phosphorylation in $CD45^-$ mutant SAKRTLS 12.1 cells, the physical association of $p56^{lck}$ with CD4 does not appear to interfere with the ability of CD45 to decrease the phosphorylation of $p56^{lck}$ on tyrosine.

Possible Regulatory Role of CD45 in T-Lymphocyte Activation. In most lymphoid cell lines, $p56^{lck}$ is phosphorylated at Tyr-505, Tyr-394, and at several unidentified serines in the amino-terminal domain (15, 26, 35). Mutagenesis suggests that phosphorylation at Tyr-505 inhibits the kinase activity of $p56^{lck}$ (15, 16). To determine whether the expression of CD45 leads to preferential dephosphorylation of either Tyr-505 or Tyr-394, tryptic peptide maps of $p56^{lck}$ were prepared from $CD45^+$ and $CD45^-$ SAKRTLS 12.1 cell lines metabolically labeled with $^{32}P_i$ (Fig. 3 *c* and *d*). The maps showed notably increased phosphorylation at Tyr-505 relative to either Tyr-394 or the unidentified serines in $p56^{lck}$ from $CD45^-$ cells versus $CD45^+$ cells. We conclude that CD45 selectively decreases phosphorylation of $p56^{lck}$ at Tyr-505.

The stoichiometry of phosphorylation of Tyr-505 in T cells has never been measured accurately. Our finding that the loss of CD45 leads to a 2- to 3-fold increase in the phosphorylation of Tyr-505 shows that this site is not fully phosphorylated in $CD45^+$ cells. This suggests that not all of the $p56^{lck}$ in T cells is normally inhibited by phosphorylation on Tyr-505 and is consistent with the idea that $p56^{lck}$ may have a significant basal enzymatic activity (26).

$p56^{lck}$, $p59^{hck}$, and $p60^{src}$ are very closely related members of the *src* family of tyrosine protein kinases. There is evidence that all three are negatively regulated by phosphorylation on a conserved tyrosine near their carboxyl terminus. Dephosphorylation of Tyr-527 in $p60^{c\text{-}src}$, the residue homologous to Tyr-505 in $p56^{lck}$, activates the protein kinase activity of $p60^{c\text{-}src}$ both *in vivo* and *in vitro* (36, 37). Mutant $p56^{lck}$, in which Tyr-505 is substituted by Phe, induces an increase in phosphotyrosine content of cellular proteins and neoplastic transformation when expressed in NIH 3T3 fibroblasts (15, 16). Similarly, mutant $p59^{hck}$ in which Tyr-501, the homologous residue to Tyr-505, is altered to Phe also induces an increase in phosphotyrosine content of cellular proteins and neoplastic transformation when expressed in fibroblasts (38). Collectively, these data strongly suggest that the protein kinase activity of $p56^{lck}$ is regulated by the opposing actions of one or more inhibitory protein kinases and activating protein phosphatases, which together determine the extent of phosphorylation of Tyr-505. The results we report here imply that CD45 may be the PTPase, or one of the PTPases, that directly or indirectly activates $p56^{lck}$. We have not yet been able to demonstrate a significant difference in the *in vitro* protein kinase activity of $p56^{lck}$ isolated from $CD45^+$ and $CD45^-$ cell lines. However, in light of previous difficulties in demonstrating increased *in vitro* protein kinase activity of mutants $p56^{lckF505}$ (16) and $p59^{hckF501}$ (38), this result was not totally unexpected. We have recently found that antibody-mediated coclustering of CD45 and CD4 inhibits an increase

in phosphorylation on tyrosine of $p56^{lck}$ induced by crosslinking of CD4 with anti-CD4 antibodies (H.L.O. and I.S.T., unpublished data). This provides independent evidence that CD45 can alter the phosphorylation state of $p56^{lck}$ *in vivo*.

The level of expression of CD45 is unlikely to be the major factor determining its regulatory effects in normal cells, as this does not change dramatically during hematopoietic development (1). It is more likely that the enzymatic activity of CD45 is subject to regulation. The extracellular domains of the CD45 isoforms may interact with a family of molecules on the surface of other cells or bind a class of soluble ligands. Interactions involving the extracellular domain that could potentially regulate the PTPase activity of the cytoplasmic domain of CD45, or bring it in juxtaposition with a specific substrate, may be dependent upon the specific isoform expressed. Additionally, the cytoplasmic domain of CD45 is phosphorylated on multiple serines (39), and changes in its phosphorylation could also play a role in the regulation of its PTPase activity.

In normal T lymphocytes, $p56^{lck}$ is complexed with either CD4 or CD8 (33, 34). These accessory molecules have been implicated in T-cell responses, and $p56^{lck}$ may, therefore, play a regulatory role in antigen-stimulated T-cell activation. If so, CD45, by virtue of its potential ability to activate $p56^{lck}$, may also be involved in signal transduction in T cells. The notion that CD45 may play a role in T-lymphocyte proliferation is supported by the recent demonstration by Pingel and Thomas (40) that a mutant mouse T-cell clone deficient in the expression of CD45 fails to proliferate in response to antigen or crosslinking by anti-CD3 monoclonal antibodies. However, as CD45 is widely expressed within the hematopoietic system, its role is unlikely to be restricted to T cells, and it will be important to identify additional substrates in other types of hematopoietic cells.

We thank Valerie Stallings for technical assistance and Tony Hunter and Bill Boyle for helpful discussions. This work was supported by grants from the National Institutes of Health. H.L.O. was supported by Damon Runyon–Walter Winchell Cancer Research Fund Fellowship (DRG-1008). D.A.S. is a Leukemia Society of America Special Fellow, and P.J. is a Fellow of the Cancer Research Institute.

1. Thomas, M. (1989) *Annu. Rev. Immunol.* **7**, 339–369.
2. Barclay, A. N., Jackson, D. I., Willis, A. C. & Williams. A. F. (1987) *EMBO J.* **6**, 1259–1264.
3. Ralph, S. J., Thomas, M. L., Morton, C. M. & Trowbridge, I. S. (1987) *EMBO J.* **6**, 1251–1257.
4. Thomas, M. L., Reynolds, P. J., Chain, A., Ben-Neriah, Y. & Trowbridge, I. S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5360–5363.
5. Saga, Y., Tung, J. S., Shen, F. W. & Boyse, E. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5364–5368.
6. Streuli, M., Hall, L. R., Saga, Y., Schlossman, S. F. & Saito, H. (1987) *J. Exp. Med.* **166**, 1548–1566.
7. Jackson, D. I. & Barclay, A. N. (1989) *Immunogenetics* **29**, 281–287.
8. Arthur, R. P. & Mason, D. (1986) *J. Exp. Med.* **163**, 774–786.
9. Akbar, A. N., Terry, L., Timms, A., Beverley, P. C. L. & Janossy, G. (1988) *J. Immunol.* **140**, 2171–2178.
10. Birkeland, M. L., Johnson, P., Trowbridge, I. S. & Pure, E. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6734–6738.
11. Charbonneau, H., Tonks, N. K., Walsh, K. A. & Fischer, E. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7182–7186.
12. Tonks, N. K., Charbonneau, H., Diltz, C. D., Fischer, E. H. & Walsh, K. A. (1988) *Biochemistry* **27**, 8696–8701.
13. Marth, J. D., Peet, R., Krebs, E. G. & Perlmutter, R. M. (1985) *Cell* **43**, 393–404.
14. Vorona, A. F. & Sefton, B. M. (1986) *Nature (London)* **319**, 682–685.
15. Marth, J. D., Cooper, J. A., King, C. S., Ziegler, S. F., Tinker, D. A., Overell, R. W., Krebs, E. G. & Perlmutter, R. M. (1988) *Mol. Cell. Biol.* **8**, 540–550.
16. Amrein, K. E. & Sefton, B. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4247–4251.
17. Martorell, J., Vilella, R., Borche, L., Rojo, I. & Vives, J. (1987) *Eur. J. Immunol.* **17**, 1447–1451.
18. Mittler, R. S., Greenfield, R. S., Schacter, B. Z., Richard, N. F. & Hoffman, M. K. (1987) *J. Immunol.* **138**, 3159–3166.
19. Ledbetter, J. A., Tonks, N. K., Fischer, E. H. & Clark, E. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8628–8632.
20. Hyman, R. & Trowbridge, I. S. (1981) *Immunogenetics* **12**, 511–523.
21. Hyman, R., Trowbridge, I. S., Stallings, V. & Trotter, J. (1982) *Immunogenetics* **15**, 413–420.
22. Johnson, P., Greenbaum, L., Bottomly, K. & Trowbridge, I. S. (1989) *J. Exp. Med.* **169**, 1179–1184.
23. Domingo, D. L. & Trowbridge, I. S. (1988) *J. Biol. Chem.* **263**, 13386–13392.
24. Trowbridge, I. S. (1978) *J. Exp. Med.* **148**, 313–323.
25. Trowbridge, I. S. & Omary, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3039–3043.
26. Hurley, T. R. & Sefton, B. M. (1989) *Oncogene* **4**, 265–272.
27. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
28. Shackelford, D. A. & Trowbridge, I. S. (1984) *J. Biol. Chem.* **259**, 11706–11712.
29. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
30. Kamps, M. P. & Sefton, B. M. (1988) *Oncogene* **2**, 305–315.
31. Kamps, M. P. & Sefton, B. M. (1989) *Anal. Biochem.* **176**, 22–27.
32. Wong, T. W. & Goldberg, A. R. (1983) *J. Biol. Chem.* **258**, 1022–1025.
33. Rudd, C. E., Trevillyan, J. M., Dasgupta, J. D., Wong, L. L. & Schlossman, S. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5190–5194.
34. Veillette, A., Bookman, M. A., Horak, E. M. & Bolen, J. B. (1988) *Cell* **55**, 301–308.
35. Veillette, A., Horak, I. D., Horak, E. M., Bookman, M. A. & Bolen, J. B. (1988) *Mol. Cell. Biol.* **8**, 4353–4361.
36. Cooper, J. A. & King, C. S. (1986) *Mol. Cell. Biol.* **6**, 4467–4477.
37. Hunter, T. (1987) *Cell* **49**, 1–4.
38. Ziegler, S. F., Levin, S. D. & Perlmutter, R. M. (1989) *Mol. Cell. Biol.* **9**, 2724–2727.
39. Omary, M. B. & Trowbridge, I. S. (1980) *J. Biol. Chem.* **255**, 1662–1669.
40. Pingel, J. T. & Thomas, M. L. (1989) *Cell* **58**, 1055–1065.