Rapid activation of the T-cell tyrosine protein kinase pp56\(^{\text{ck}}\) by the CD45 phosphotyrosine phosphatase

(\text{lk} / \text{CD45/tyrosine phosphorylation/T lymphocyte})

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ABSTRACT T lymphocytes express a tyrosine protein kinase (TPK; protein-tyrosine kinase; ATP:protein-tyrosine O-phosphotransferase, EC 2.7.1.112), pp56\(^{\text{ck}}\) that is encoded by the \text{lk} protooncogene. This TPK was recently found to be associated with the intracellular domain of the T-cell surface glycoproteins, CD4 and CD8, suggesting that it plays an important role in T-cell development and activation. We have studied the regulation of pp56\(^{\text{ck}}\) and found that this kinase can be rapidly activated by an endogenous mechanism present in T-lymphocyte membranes. This activation was sensitive to sodium orthovanadate and O-phosphotyrosine, consistent with the involvement of a phosphotyrosine phosphatase (PTPase; protein-tyrosine-phosphatase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) in pp56\(^{\text{ck}}\) activation. Based on a recent report demonstrating that CD45, the leukocyte common antigen, is a membrane-bound PTPase, we analyzed its role in pp56\(^{\text{ck}}\) activation. CD45 was found to be the major (\textgreater 90\%) PTPase in membranes of the murine T-lymphoma line BW5147. Moreover, activation of pp56\(^{\text{ck}}\) was undetectable in a mutant BW5147 line lacking CD45 expression (and the associated PTPase activity). In contrast, activation of pp56\(^{\text{ck}}\) was readily detected in the wild-type lymphoma line. More important, when immunoprecipitated CD45 was added to pp56\(^{\text{ck}},\) the TPK activity of the latter increased \textgreater 2-fold within minutes. This effect of CD45 was completely blocked by sodium orthovanadate. These findings indicate an important role for the CD45 PTPase in pp56\(^{\text{ck}}\) activation. This role could be mediated by direct dephosphorylation of a regulatory tyrosine residue in pp56\(^{\text{ck}}\).

Phosphorylation of proteins on tyrosine residues plays a crucial role in the regulation of cell proliferation and differentiation (1). To date, at least 25 different mammalian tyrosine protein kinases (TPKs; protein-tyrosine kinase; ATP: protein-tyrosine O-phosphotransferase, EC 2.7.1.112) or putative TPKs have been identified (2–4). The src family of cellular protooncogenes (c-src, c-yes, c-fgr, fyn, lyn, lck, hck, and \text{tkl}) encodes nonreceptor TPKs devoid of transmembrane or extracellular domains. Nevertheless, these kinases are located at the plasma membrane, perhaps by virtue of a membrane-anchored myristic acid covalently attached to their N terminus (5–7).

The TPK encoded by the \text{lk} protooncogene, pp56\(^{\text{ck}},\) is expressed at high levels only in T lymphocytes (8). It was first detected in the Moloney murine leukemia virus-induced T-cell lymphoma line LSTRA (7, 9, 10), which overexpresses the \text{lk} gene due to insertion of viral promoter sequences. As a result, the corresponding mRNA is transcribed and translated more efficiently, and the amount of pp56\(^{\text{ck}}\) is \textasciitilde 50-fold higher than in other T-cell lines (11). The \text{lk} gene was cloned and sequenced from LSTRA and murine thymocytes (8), and later from human T lymphocytes (12, 13).

pp56\(^{\text{ck}}\) is highly phosphorylated \textit{in vivo} on a tyrosine residue (Tyr-505) in its C terminus (14). Mutation of this residue to phenylalanine increases the TPK activity and evokes the transforming potential of the protein (15, 16). Hence, Tyr-505 apparently is involved in suppression of transforming activity. All members of the src family have an analogous C-terminal tyrosine residue implicated in negative regulation of this activity (17). Phosphorylation of Tyr-505 appears not to be mediated by pp56\(^{\text{ck}}\) itself; instead, pp56\(^{\text{ck}}\) autophosphorylates another tyrosine residue, Tyr-394 (14, 15). This tyrosine corresponds to the major autophosphorylation site in pp60\(^{\text{src}}\) and other TPKs. Phosphorylation of Tyr-394 correlates with catalytic activity toward exogenous substrates (15, 18).

Recently, pp56\(^{\text{ck}}\) was found to be physically associated with the cytoplasmic domain of the CD4 and CD8 glycoproteins in T cells (19, 20). These two surface molecules play an important role in T-cell interactions with antigen-presenting cells (21–23) and in T-cell receptor repertoire selection in the thymus (24–26), suggesting a role for pp56\(^{\text{ck}}\) in these T-lymphocyte-specific processes (27).

In this paper, we show that pp56\(^{\text{ck}}\) can be rapidly activated in isolated T-lymphocyte membranes by an endogenous mechanism. This activation was blocked by sodium orthovanadate (Na\(_3\)VO\(_4\)) or O-phosphotyrosine, suggesting the action of a phosphotyrosine phosphatase (PTPase; protein-tyrosine-phosphatase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48). Indeed, in a mutant T lymphoma lacking the major membrane-bound PTPase, CD45 (T200, leukocyte common antigen), no activation of pp56\(^{\text{ck}}\) could be obtained under conditions in which the wild-type cells demonstrated a clear response. In addition, we show that isolated CD45 can directly activate pp56\(^{\text{ck}}\) in mixed immunoprecipitates of the two proteins. Our results suggest an important role for CD45 in regulation of pp56\(^{\text{ck}}\) activity in T cells.

MATERIALS AND METHODS

Cells. Human peripheral blood lymphocytes (PBLs; \textgeq 80\% T lymphocytes) were isolated from the blood of healthy volunteers by gradient centrifugation on Histopaque (Sigma). LSTRA cells were kept at logarithmic growth in RPMI 1640 medium with 10% fetal calf serum and BW5147 cells [wild type or a CD45\(^{-}\) mutant obtained from R. Hyman (Salk Institute, San Diego)] in Dulbecco's modified Eagle's medium with 10% horse serum (both sera from Flow Laboratories).

Membrane Preparation. A membrane fraction was prepared by sonicating cells in a hypotonic lysis buffer [25 mM Tris-HCl, pH 7.5, 25 mM sucrose/0.1 mM EDTA/5 mM MgCl\(_2\)/5 mM dithiotreitol/1 mM phenylmethylsulfonyl fluo-

Abbreviations: PBL, peripheral blood lymphocyte; PTPase, phosphotyrosine phosphatase; TPK, tyrosine protein kinase.

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Tyrosine Kinase Assay. Phosphorylation of a synthetic peptide, Lys-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-(Johnson & Johnson Biotechnology Center, San Diego), corresponding to the major autophosphorylation site of pp56\(^{ck}\), was measured as described by Casnelli et al. (9). The assay mixture contained 50 mM Hapes (pH 7.5), 10 mM MgCl\(_2\), 1 mM MgCl\(_2\), 1 mM Na\(_3\)VO\(_4\), 50 \(\mu\)M \(\gamma\)-\(\text{32P}\)ATP (ICN specific activity, \(\approx 5\mu\text{Ci/mmol}\); CI = 37 GBq), 10 mM substrate, and 10–15 \(\mu\)g of membrane protein in a final vol of 25 \(\mu\)l. In some experiments, the membrane protein was incubated at 30°C for various times (30 sec to 5 min) in 10 \(\mu\)l of lysis buffer with or without Na\(_3\)VO\(_4\) (1 mM), prior to initiating the kinase assay by adding assay mixture and \(\gamma\)-\(\text{32P}\)ATP in 15 \(\mu\)l. After 2 min at 30°C, the reaction was stopped by addition of 155 \(\mu\)l of 3.2% trichloroacetic acid and 20 \(\mu\)l of bovine serum albumin (10 mg/ml). After 30 min on ice, the mixture was centrifuged for 10 min at 13,000 \(\times\) g, and 100 \(\mu\)l of the supernatant was applied to phosphocellulose paper. The radioactivity present in the paper after six or seven washes in 75 mM H\(_2\)PO\(_4\) was assayed by liquid scintillation counting. Essentially identical results were obtained with a different peptide (Lys-Arg-Leu-Ile-Glu-Asp-Asn-Glu-Tyr-Thr-Ala-(Johnson & Johnson Biotechnology Center, San Diego)) in which the threonine residue was replaced by an alanine to exclude possible threonine phosphorylation. pp56\(^{ck}\) bound to CD4 was immunoprecipitated with OKT4 (monoclonal antibody against CD4) from detergent lysates of PBLs (19) and was assayed for TPK activity as described above. CD45 was similarly immunoprecipitated with the monoclonal antibody GAP 8.3 (obtained from the American Type Culture Collection) from PBLs lysed in 0.2% Nonidet P-40, phosphate-buffered saline, aprotinin (10 \(\mu\)g/ml), and leupeptin (10 \(\mu\)g/ml).

Autophosphorylation of pp56\(^{ck}\). Autophosphorylation of membranes was carried out in a final vol of 100 \(\mu\)l containing 500 \(\mu\)g of protein, 25 mM Hapes (pH 7.4), 10 mM MgCl\(_2\), 10 mM MnCl\(_2\), 140 \(\mu\)Ci of \(\gamma\)-\(\text{32P}\)ATP, and 1 mM Na\(_3\)VO\(_4\). The mixture was incubated at 30°C for 2 min and the reaction was stopped by adding 10 \(\mu\)l of 5% SDS/0.5 M Tris-HCl, pH 8.0/2.0 mM EDTA, and heating the mixture to 95°C for 1 min. Four hundred microliters of 1.25% Triton X-100/15% sodium deoxycholate/150 mM sodium chloride/0.5 M Tris-HCl, pH 8.0/2.0 mM EDTA/1 mM Na\(_3\)VO\(_4\) was added, and the mixture was centrifuged for 15 min at 13,000 \(\times\) g at +4°C (7). The supernatant was precleared with 50 \(\mu\)l of formalin-fixed Staphylococcus aureus (strain Cowan I) and incubated for 4 hr with 5 \(\mu\)l of a polyclonal rabbit anti-pp56\(^{ck}\) antiserum (gifts from B. Selfon and R. Perlmutter). Immunocomplexes were collected with 100 \(\mu\)l of the fixed staphylococci, washed several times in lysis buffer, and analyzed on 7.5% SDS/polyacrylamide gels.

PTPase Assay. The enzyme(s) was assayed according to Leis and Kaplan (28) at 37°C in a 50-\(\mu\)l vol containing 10 mM \(O\)-phosphotyrosine as a substrate, 1 mM EDTA, 100 mM sodium acetate (pH 6.0), and 2–10 \(\mu\)g of membrane protein. The reaction was stopped by the addition of 150 \(\mu\)l of 25% trichloroacetic acid followed by 50 \(\mu\)l of bovine serum albumin (10 mg/ml). Precipitated protein was removed by centrifugation and the supernatants were used for measurement of liberated inorganic phosphate as described (29) by using a mixture of perchloric acid, ammonium molybdate, and ascorbic acid, heating the samples at 36°C for 15 min, and measuring the absorbance at 750 nm. KH\(_2\)PO\(_4\) was used as standard.

Immunofluorescence. BW5147 cells (wild type or CD45 \(^{-}\) mutant) were washed and treated for 40 min on ice with an optimal dilution of a mixture of culture supernatants from two hybridomas producing monoclonal anti-murine CD45 antibodies, M1/9.3.4.HL.2 (ATCC no. TIB122) and M1/89.18.7 (ATCC no. TIB124). After additional washes, the cells were incubated for 5 min on ice with a 1:25 dilution of a fluorescein-labeled affinity-purified F(ab')\(_2\) fragment of a mouse anti-rat IgG (Jackson ImmunoResearch), washed, and resuspended in phosphate-buffered saline supplemented with 2% fetal bovine serum and 0.02% sodium azide. Cells were analyzed on a FACScan fluorescence-activated cell sorter.

RESULTS

During initial studies, we found that PBL membranes isolated at +4°C in lysis buffer lacking Na\(_3\)VO\(_4\) displayed a consistent 20–30% increase in tyrosine kinase activity compared to membranes isolated in the presence of Na\(_3\)VO\(_4\) (data not shown). Furthermore, when PBL membranes isolated in vanadate-free buffer were kept at 30°C for short times prior to the kinase assay (containing Na\(_3\)VO\(_4\)), there was a time-dependent increase in their tyrosine kinase activity (Fig. 1). The activation reached a plateau in 1–2 min and was \(\approx 2\)-fold. When membranes prepared in Na\(_3\)VO\(_4\)-containing buffer were treated similarly, only a marginal and transient activation was seen. Similar results (data not shown) were found when the T lymphocytes used for membrane preparation were further enriched by passage through nylon wool columns (30).

To determine whether activation of pp56\(^{ck}\) contributed to the rapid increase in membrane-bound kinase activity, we quantitated TPK activity in membranes from LSTRA cells, which are known to express \(\approx 50\)-fold higher levels of pp56\(^{ck}\) protein than other T cells (11). As in the case of PBL membranes, a rapid time-dependent increase in kinase activity, which was sensitive to Na\(_3\)VO\(_4\), was clearly evident (Fig. 2), despite the fact that the basal tyrosine kinase activity in LSTRA membranes was \(\approx 65\) times higher than in human T-cell membranes. This result indicates that pp56\(^{ck}\) is rapidly activated under our experimental conditions. If a TPK other than pp56\(^{ck}\) was responsible for the increase in kinase activity, this increase would most likely be masked by the high pp56\(^{ck}\) activity in LSTRA cells.

To further investigate possible changes in the specific activity of pp56\(^{ck}\), we incubated lymphocyte membranes

Fig. 1. Effects of short preincubations without ATP at 30°C on the tyrosine kinase activity of human T-cell membranes isolated in the presence (○) or absence (□) of Na\(_3\)VO\(_4\).
with [γ-32P]ATP and divalent cations and analyzed the extent of pp56<sup>ck</sup> autophosphorylation by immunoprecipitation. When the membranes were isolated in Na<sub>3</sub>VO<sub>4</sub>-free buffer and kept at 30°C for 2 min prior to addition of radiolabeled ATP, there was a severalfold increase in subsequent autophosphorylation (Fig. 3). Addition of 1 mM Na<sub>3</sub>VO<sub>4</sub> during the preincubation step ablated this increase.

Since Na<sub>3</sub>VO<sub>4</sub> is a known PTPase inhibitor, we wished to determine whether the membranes contain this activity. Direct measurements confirmed the presence of a highly active PTPase in the T-cell membranes (Fig. 4 A and B). Using free O-phosphotyrosine as a substrate, this enzyme had an acidic pH optimum (Fig. 4C) and a specific activity of ≈0.4 μmol·min<sup>-1</sup>·mg<sup>-1</sup>. The enzyme(s) was inhibited by Na<sub>3</sub>VO<sub>4</sub> in a dose-dependent manner (Fig. 4D), with half-maximal inhibition at ≈300 μM. Free O-phosphotyrosine (10 mM) was also an inhibitor of the increase in tyrosine kinase activity of membranes kept at 30°C (data not shown), probably acting as a competing substrate for the phosphatase. Phosphoserine and phosphothreonine were less efficient in this respect.

![Fig. 2.](image)

**Fig. 2.** Effects of short preincubations without ATP at 30°C on the tyrosine kinase activity of LSTRA membranes isolated in the presence (●) or absence (○) of Na<sub>3</sub>VO<sub>4</sub>.

![Fig. 3.](image)

**Fig. 3.** Autophosphorylation of pp56<sup>ck</sup> analyzed by immunoprecipitation from human T-lymphocyte membranes isolated in the presence of Na<sub>3</sub>VO<sub>4</sub>, no preincubation (lane a); in the absence of Na<sub>3</sub>VO<sub>4</sub>, no preincubation (lane b); in the presence of Na<sub>3</sub>VO<sub>4</sub> and preincubated for 2 min at 30°C (lane c); in the absence of Na<sub>3</sub>VO<sub>4</sub> and preincubated for 2 min at 30°C (lane d).

![Fig. 4.](image)

**Fig. 4.** PTPase activity in T-lymphocyte membranes. (A and B) Linearity of the assay with protein concentration and time, respectively. (C) pH optimum. (D) Sensitivity of Na<sub>3</sub>VO<sub>4</sub>.

Recent studies documented that CD45, the leukocyte common antigen, is a membrane-bound PTPase in human spleen (31). We therefore wished to determine whether the CD45-associated PTPase activity plays a role in activating pp56<sup>ck</sup>. Thus, we analyzed pp56<sup>ck</sup> activation in the BW5147 (T200<sup>a</sup>) 5.1 cell line, which is a CD45<sup>−</sup> mutant of the BW5147 murine T lymphoma (Fig. 5A). This mutant was derived by repeated cytotoxic elimination of CD45<sup>+</sup> cells using anti-CD45 antibodies plus complement (32). We detected very low (<10%) PTPase activity in membranes of this CD45<sup>−</sup> mutant compared to the wild-type BW5147 (Fig. 5B), indicating that in wild-type BW5147 cells, >90% of the membrane-bound PTPase activity is due to CD45. When these membranes were kept at 30°C for short periods of time prior to measuring their tyrosine kinase activity, no changes in kinase activity were

![Fig. 5.](image)

**Fig. 5.** (A) Expression of CD45 in (T200<sup>−</sup>)BW5147 and in wild-type BW5147 cells measured by immunofluorescence staining and FACS analysis. (B) PTPase activity in membranes of (T200<sup>−</sup>)-BW5147 (○) and wild-type BW5147 cells (●).
Fig. 6. Effects of short preincubations without ATP at 30°C on the tyrosine kinase activity of (T200′/BW5147 membranes (c)) and on wild-type BW5147 membranes (d).

detected in the CD45− mutant, while a rapid 2-fold activation was evident in wild-type BW5147 membranes (Fig. 6). These results show that, in the absence of the CD45 phosphatase, activation of pp56⁰lck (and possibly other TPKs) does not occur. The <10% PTPase activity in the CD45− mutant could be due to other PTPases or, simply, to assay background (i.e., spontaneous release of P from membranes). At any rate, this residual activity clearly does not play any role in pp56⁰lck activation (Fig. 6).

Since the selection process of the CD45− BW5147 mutant could affect expression of other proteins in addition to CD45, we performed an additional experiment to determine whether CD45 acts directly on pp56⁰lck. The two proteins were immunoprecipitated from human peripheral blood T cells separately, mixed, and incubated at 30°C prior to TPK activity measurement. Under these conditions, the TPK activity of pp56⁰lck increased ~2.5-fold in 3 min (Table 1). The activation was sensitive to Na3VO4 and was absent when control antibodies were used instead of the anti-CD45 antibody, GAP 8.3. The CD45 immunoprecipitates did not contain any TPK activity (Table 1) but had a PTPase activity, which accounted for a substantial part of the overall PTPase activity in membrane preparations. In contrast, immunoprecipitates of control antibodies did not contain any detectable PTPase activity (T.M. and A.A., unpublished data). Due to the difficulty in completely solubilizing PTPase activity from membranes (31), it is not possible to conclude from these experiments what proportion of the membrane-bound activity is accounted for by CD45.

### DISCUSSION

Our results show that an endogenous mechanism for the rapid activation of the T-cell TPK, pp56⁰lck, exists in T-lymphocyte membranes. This activation was documented in two murine T-lymphocyte lines [LSTAR (2) and BW5147 (6)], in human PBLs (Figs. 1 and 3, Table 1), and in human HPB-MLT leukemic cells (unpublished data), indicating that it is a general event occurring in different types of T cells. The sensitivity of this mechanism to Na3VO4 and o-phosphoryrosine suggests the involvement of a PTPase acting on a regulatory phosphotyrosine residue. One likely candidate to mediate this activation is CD45, which is expressed at high levels on all leukocytes and was recently found to have extensive homology, in its intracellular domain, to a major PTPase purified and molecularly cloned from human placenta (33). It was further shown that CD45 is indeed a PTPase (31). The findings that the activation of pp56⁰lck by Na3VO4-sensitive PTPase is absent in cells lacking CD45, and that this PTPase normally represents >90% of membrane-bound Na3VO4-sensitive PTPase activity, strongly suggest that CD45 could itself be the PTPase acting on pp56⁰lck. It should be possible to confirm this notion by transfection of the CD45 gene into the CD45− BW5147 mutant cells.

To directly demonstrate such a role for CD45, we reconstituted isolated pp56⁰lck with CD45 prepared from a separate batch of cells. In these experiments, CD45, but not control, immunoprecipitates stimulated a 2.5-fold increase in TPK activity. Moreover, as in the case of intact membranes, this activation was abolished by Na3VO4. These results demonstrate that the CD45 PTPase can directly mediate pp56⁰lck activation.

All members of the src family of TPKs have a conserved tyrosine residue near their C-terminal end, which is strongly implicated in regulating their catalytic activity (17). This residue (Tyr-505 in pp56⁰lck) is heavily phosphorylated in vivo (14, 15, 17). In parallel, the catalytic activity of the enzyme is low. In contrast, the lack of this tyrosine residue in the corresponding oncogenic retroviral forms (v-src, v-yes, v-fgr) is associated with much higher kinase and transforming activities (34). Consistent with the proposed suppression of kinase activity by phosphorylation of the C-terminal tyrosine are the findings that mutations of lck (15, 16) or fyn (35), which replace this tyrosine, elevate the kinase activity and evoke the transforming potential of these genes. Also, treatment of pp60⁰src immunoprecipitates with phosphatase increases the activity of the kinase (36).

The factors influencing phosphorylation of the C-terminal tyrosine are poorly understood. In the case of pp60⁰src, this event is not a result of autophosphorylation and does not require membrane localization (37), suggesting that a distinct, presumably cytosolic TPK phosphorylates the C-terminal tyrosine residue, thereby suppressing the activity of the src family kinases. Only three TPKs—c-abl, c-fes/fps, and c-pim1 (38)—are presently known to be cytosolic. Of these, c-abl and c-pim1 are expressed in lymphoid cells (39, 40). Our experiments were designed to keep the kinase phosphorylating Tyr-505 of pp56⁰lck inactive by omitting ATP during preincubation of the membranes. Furthermore, if the Tyr-505 kinase is cytosolic, it would be largely lost during membrane preparation.

In addition to the lck gene, at least two other src family members are expressed in lymphocytes—namely, fyn (20) in T lymphocytes and sck (3) in spleen. Our data do not exclude the possibility that these two TPKs are activated together.

### Table 1. TPK activity in mixed immunoprecipitates

<table>
<thead>
<tr>
<th>Immunoprecipitating antibody</th>
<th>Na3VO4 (1 mM)</th>
<th>TPK activity, % of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okt4</td>
<td>−</td>
<td>100</td>
</tr>
<tr>
<td>GAP 8.3</td>
<td>−</td>
<td>20.1 ± 2.1 (n = 4)</td>
</tr>
<tr>
<td>Okt4 + GAP 8.3</td>
<td>−</td>
<td>100.1 ± 10.4 (n = 5)</td>
</tr>
<tr>
<td>Okt4 + Okt3</td>
<td>−</td>
<td>99.1 (n = 1)</td>
</tr>
<tr>
<td>Okt4 + 142</td>
<td>−</td>
<td>103.5 (n = 1)</td>
</tr>
</tbody>
</table>

pp56⁰lck was immunoprecipitated with Okt4, a monoclonal anti-CD4 antibody, from 20 × 10⁶ PBLs as described (19), mixed with CD45 or controls immunoprecipitated from 5 × 10⁶ PBLs. Samples were kept at 30°C for 3 min prior to TPK activity measurement. The TPK activity of the Okt4 immunoprecipitates was 426.3 ± 108.3 fmol per min per 10⁶ cells (n = 11). GAP 8.3 is an anti-CD45 monoclonal antibody. Negative controls included monoclonal antibodies Okt3 (anti-CD3) or 142-24E5 (specific for ras-related proteins expressed in T cells).
with pp56kck under our experimental conditions. In LSTRA cells, however, this possible activation of the \( \text{fyn} \) and \( \text{tik} \) kinases would most likely escape detection due to the \( \sim 50 \)-fold higher than normal levels of pp56kck in this cell line (11). The fact that a nearly 2-fold increase in tyrosine kinase activity was readily detected in LSTRA membranes indicates that this increase reflects the selective, if not exclusive, activation of pp56kck. However, direct quantitation of the relative contribution of pp56kck to the overall TPK activity in intact cells is not possible from immunodepletion experiments since such experiments require membrane solubilization with detergents. Under these conditions, the detergent was found to stimulate the TPK activity (T.M., unpublished observation).

The role of pp56kck in T-cell physiology is still poorly understood. Our results show that an intrinsic mechanism for activation of pp56kck, apparently involving CD45, exists in T cells. Since pp56kck is believed to be regulated by phosphorylation of a C-terminal tyrosine (Tyr-505) and CD45 is a PTase, we consider a direct dephosphorylation of Tyr-505 by CD45 to be the most likely mechanism underlying the activation of pp56kck in our experiments. Taken together with the findings that pp56kck is associated with the CD4 or CD8 glycoproteins (19, 20), these findings would suggest a hypothetical mechanism for regulation of tyrosine phosphorylation in T lymphocytes. In this model, pp56kck is activated when CD45 and the pp56kck-associated CD4 or CD8 are brought together—e.g., by cross-linking. This concept is supported by the recent finding that the increase in intracellular free Ca\(^{2+}\) concentration induced by antibody-mediated cross-linking of CD4 was strongly amplified when CD45 was coupled to CD4 (41). Thus, extracellular events such as binding of CD4/CD8 and/or CD45 to their ligands could potentially regulate the intracellular tyrosine phosphorylation of proteins in T lymphocytes by restricting the lateral movement of CD4/CD8 and CD45 in the plasma membrane. The association of CD45 with the cytoskeletal protein fodrin (42) could be important in determining the mobility of CD45. Whether CD45 interacts with pp56kck under certain physiological conditions, such as T-cell activation or thymic development, remains to be determined.

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