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Differential Effects of Expression of the CD45 Tyrosine Protein Phosphatase on the Tyrosine Phosphorylation of the *lck*, *fyn*, and c-*src* Tyrosine Protein Kinases

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Expression of the CD45 tyrosine protein phosphatase is required for the response of functional lymphocytes to stimulation through the antigen receptor. One or more of its substrates may therefore be essential for signal transduction during lymphocyte activation. We have studied the phosphorylation of the closely related *lck*, *fyn*, and c-*src* tyrosine protein kinases in leukemic murine T-cell lines that have lost the expression of CD45. The phosphorylation of the *lck* kinase at an inhibitory site of tyrosine phosphorylation, Tyr-505, was increased by two-, six-, and eightfold in three different cell lines. Phosphorylation of the *fyn* kinase at the homologous site, Tyr-531, was unaltered in one of these cell lines, but increased by 2.5-fold in the two others. The phosphorylation of $p60^{c\text{-}src}$ at the homologous tyrosine was essentially unchanged in the one CD45-negative cell line in which it was examined. The expression of CD45 therefore regulates the phosphorylation and potentially the activity of the *lck* and *fyn* tyrosine protein kinases, but the effect on the *lck* kinase is much greater than on the *fyn* kinase. This finding and the observation that CD45 had no effect on the phosphorylation of $p60^{c\text{-}src}$ suggest that CD45 exhibits polypeptide substrate specificity in vivo. Additionally, these findings are consistent with the hypothesis that the unresponsiveness of CD45-negative lymphoid cells to antigenic stimulation is due largely to hyperphosphorylation of the *lck* kinase.

Expression of the CD45 tyrosine protein phosphatase on the surface of helper and cytotoxic T cells is essential for the response of such cells to signalling through the antigen receptor (18, 19, 31, 39). This argues strongly that the dephosphorylation of one or more proteins on tyrosine is required for cellular responsiveness to antigen. There is also evidence from work with inhibitors of tyrosine protein kinases that tyrosine protein phosphorylation is required for signalling through the T-cell antigen receptor (15, 26). Although these findings might seem contradictory, they can be reconciled if the substrate(s) of CD45 is a tyrosine protein kinase that is activated through dephosphorylation by CD45.

The *src* family tyrosine protein kinases are inhibited by phosphorylation of a conserved site near their carboxy termini—Tyr-527 in $p60^{c\text{-}src}$, Tyr-505 in $p56^{lck}$, and Tyr-531 in $p59^{fyn}$ (7)—and are activated by dephosphorylation of this site. Since the *lck*, *fyn*, and *yes* kinases are expressed in T lymphocytes, they are potential substrates of CD45 that could play a role in T-cell activation. Roles for the *lck* and *fyn* kinases in T-cell activation and maturation are suggested by other findings. The *lck* kinase is bound to the cytoplasmic tails of CD4 and CD8 cell surface glycoproteins (32, 37) that function as coreceptors with the T-cell antigen receptor. Binding to the *lck* kinase is essential for the ability of CD4 and CD8 to augment the response of T cells to antigen (8, 23, 40). Additionally, an activated form of the *lck* kinase can both increase the responsiveness of some T-cell hybridomas to antigen (1) and induce antigen-independent production of interleukin 2 by others (21). Finally, mice lacking a functional *lck* gene are greatly impaired in the production of T lymphocytes (25), and variants of the Jurkat human T-cell line and the CTLL-2 murine T-cell line that do not express $p56^{lck}$ exhibit reduced responsiveness to stimulation through the T-cell receptor (17, 36). The *fyn* kinase can be isolated in association with the T-cell antigen receptor (34), implying a role for the protein in signal transduction. Transgenic mice expressing abnormally high levels of the *fyn* protein produce T lymphocytes that show increased responsiveness to activation (6). Correspondingly, T lymphocytes from mice expressing high levels of an inactive *fyn* kinase and thymocytes from mice in which *fyn* is not expressed show a reduced response to stimulation through the T-cell receptor (3, 35).

We have shown previously that the loss of expression of the CD45 phosphatase is correlated with increased phosphorylation of the *lck* kinase at its site of inhibitory phosphorylation, Tyr-505, in the SAKRTLS12.1 cell line (28). This suggested that CD45 is a natural activator of the *lck* kinase and raised the possibility that the reason cells lacking CD45 were unresponsive to stimulation through the antigen receptor was that the *lck* kinase was inhibited by increased phosphorylation of Tyr-505. $p59^{fyn}$ can serve as a substrate of, and be regulated by, CD45 in vitro (27). To determine whether the phosphorylation of the *fyn* kinase is also regulated by the expression of CD45 in vivo, we have examined and compared the phosphorylation of the *lck* and *fyn* kinases in pairs of closely related CD45-positive and -negative murine leukemic cell lines. Additionally, to look more thoroughly at the polypeptide substrate specificity of CD45, we expressed murine $p60^{c\text{-}src}$ in one of these pairs of CD45-positive and -negative murine leukemic cell lines by way of retroviral infection and examined its phosphorylation.

MATERIALS AND METHODS

Cell lines. The CD45-negative mutant SAKRTLS12.1, BW5147, and NZB.1 murine T-lymphoma cell lines were

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derived from mutagenized parental cell lines by immunoselection with anti-CD45 antibody and complement (13, 28).

Infection of cells. A construct encoding murine c-*src* (pneoΔp60wt, a kind gift from Martin Broome) and a viral helper plasmid, SV-Ψ⁻-A-MLV (generously provided by N. Landau [24]), were introduced into 2.5×10^5 COSm-6 cells on a 5-cm plate by calcium phosphate-mediated transfection (9) as previously described (11), except that the cells were not treated with dimethyl sulfoxide but rather were incubated for 24 h in medium containing 5 μM chloroquine. At 48 h posttransfection, 5×10^5 CD45-positive and CD45-negative SAKRTLS12.1 cells were cocultivated for 24 h with the transfected cells in 2.5 ml of Dulbecco-Vogt modified Eagle's medium (DMEM) supplemented with 10% horse serum. The SAKRTLS12.1 cells were then transferred to a fresh dish containing 2.5 ml of fresh medium and incubated for 24 h. The cells were then expanded in medium containing 1 mg of the drug G418 (GIBCO/BRL) per ml into 23 wells of a 24-well plate. G418-resistant pools were screened by Western blotting (immunoblotting) with anti-p60^{src} antibodies (monoclonal antibody 327), and a pool of CD45-positive and CD45-negative SAKRTLS12.1 cells expressing high levels of p60$^{c\text{-}src}$ was used for further study.

Biosynthetic labeling and immunoprecipitation. A total of 2×10^7 cells were washed three times in phosphate-free DMEM and then incubated in 3 ml of phosphate-free DMEM supplemented with 10% phosphate-free fetal bovine serum and 1 to 1.67 mCi of $^{32}P_i$ (as $H_3{}^{32}PO_4$ [ICN]) per ml. After 6 h, cells were washed and lysed by boiling in 0.5% sodium dodecyl sulfate (SDS)–50 mM Tris-HCl (pH 8.0)–1 mM dithiothreitol as previously described (12). The lysate was then converted to RIPA (radioimmunoprecipitation assay) buffer (34a) by dilution with 5 volumes of RIPA buffer lacking SDS. After dilution, the final concentration of the lysate was 10^7 cells per ml. Rabbit polyclonal antibodies to p56^{lck} (12) and p59^{fyn} (*fyn*-2, a kind gift of Sara Courtneidge and R. Kypta) and tissue culture supernatant from the monoclonal antibody 2037D10 (anti-p60^{src}) were preadsorbed to Pansorbin (Calbiochem). Lysates were incubated sequentially with either anti-p59^{fyn} and then anti-p56^{lck} or anti-p56^{lck} and then anti-p60^{src} Pansorbin complexes, and these immune complexes were washed in RIPA buffer. Proteins were fractionated by SDS-polyacrylamide gel electrophoresis and transferred electrophoretically to Immobilon-P (Millipore) for phosphoamino acid analysis or nitrocellulose (Schleicher & Schuell) for CNBr cleavage analysis.

Labeling by autophosphorylation. Proteins to be labeled by autophosphorylation were isolated by immunoprecipitation from unlabeled cells. The immunoprecipitates were resuspended in 20 μl of 0.01 M sodium piperazine-*N*,*N*′-bis[2-ethanesulfonic acid (PIPES; pH 7.0)–0.01 M $MnCl_2$ containing 10 μCi of [γ-^{32}P]ATP (5,400 Ci/mmol; ICN) and incubated for 10 min at 30°C. The reaction was stopped by the addition of 0.5 ml of 0.01 M Tris-HCl (pH 7.2) at 4°C–0.1% Nonidet P-40 and recovered by centrifugation.

Phosphoamino acid analysis. ^{32}P-labeled proteins bound to Immobilon-P membrane were hydrolyzed with 5.7 N HCl, and the resulting phosphoamino acids were resolved by using two-dimensional electrophoresis as previously described (16).

CNBr cleavage. ^{32}P-labeled proteins bound to nitrocellulose were incubated with 50 mg of CNBr per ml in 70% formic acid for 1.5 h as previously described (20). The yields of CNBr fragments were quantified by measurement of Cerenkov radiation, and approximately equal amounts of radioactivity from each pair of CD45-positive and -negative cell lines were analyzed. The peptides were fractionated on a 24% polyacrylamide gel by utilizing a Tricine cathode buffer as previously described (20). For phosphoamino acid analysis of CNBr peptides, peptides were transferred electrophoretically to Immobilon-P and subjected to acid hydrolysis as described above.

Analysis of CNBr peptides. CNBr-cleaved peptides were visualized by autoradiography, and the resulting film was scanned with an LKB scanning densitometer. The peptide bands were then analyzed with the Hoeffer GS370 integration program, and integration unit values were determined for each peak. For p59^{fyn} and p56^{lck} from each set of mutant and parental lines, the integration units in the amino-terminal fragments were normalized to each other, and the integration units in the other peptides were adjusted accordingly. The ratio of the integration units of the carboxy-terminal fragments of p59^{fyn} (and p56^{lck}) from the CD45-negative mutant line versus the parental line for each set of lines was then calculated. The same method was used to analyze the CNBr-generated peptides from p60$^{c\text{-}src}$.

RESULTS

Tryptic peptide mapping and phosphoamino acid analysis have shown previously that p56^{lck} undergoes increased phosphorylation at Tyr-505 in CD45-negative SAKRTLS 12.1 cells (28). To examine whether the expression of CD45 affected the tyrosine phosphorylation of another *src* family member, p59^{fyn}, we examined the phosphorylation of p59^{fyn} in these cells and CD45-negative variants of two other murine T-cell lines, BW5147 and NZB.1.

Phosphoamino acid analysis of p59^{fyn} from the three pairs of CD45-positive and -negative cell lines showed the overall level of tyrosine phosphorylation in p59^{fyn} increased approximately twofold in the CD45-negative mutant derived from NZB.1 cells (Fig. 1) but was unchanged in the corresponding CD45-negative mutants derived from BW5147 and SAKRTLS12.1 cells. These effects were considerably less dramatic than what was seen with p56^{lck}, in which a marked increase in the ratio of phosphotyrosine to phosphoserine was apparent in the CD45-negative SAKRTLS12.1 cells (Fig. 1).

The use of tryptic peptide mapping with p59^{fyn} for the estimation of the extent of tyrosine phosphorylation of specific residues was not productive because it was difficult to identify the tryptic peptide containing Tyr-531. We therefore turned to CNBr mapping. As is the case with p60^{src} (4, 14) and p56^{lck} (20, 38), CNBr hydrolysis is predicted to cleave p59^{fyn} into three relatively large fragments that can be analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis: a 21-kDa fragment containing residues 2 to 190, a 9-kDa fragment containing residues 384 to 469, and a 4-kDa fragment containing residues 502 to 537 (Fig. 2a). The 9-kDa fragment would contain the autophosphorylation site, Tyr-420, and the 4-kDa fragment would contain the site of inhibitory phosphorylation, Tyr-531. As predicted, gel analysis of CNBr-generated fragments from p59^{fyn} labeled biosynthetically with $^{32}P_i$ revealed three phosphorylated fragments (Fig. 2b). Phosphoamino acid analysis of the 21-kDa amino-terminal fragment showed that it contained only phosphoserine and phosphothreonine (Fig. 2b). The 9- and 4-kDa fragments contained only phosphotyrosine (Fig. 2b). As expected for the fragment predicted to contain Tyr-420, the 9-kDa fragment was labeled extensively when p59^{fyn} was allowed to undergo autophosphorylation in vitro in the presence of [γ-^{32}P]ATP. The 4-kDa fragment was not labeled

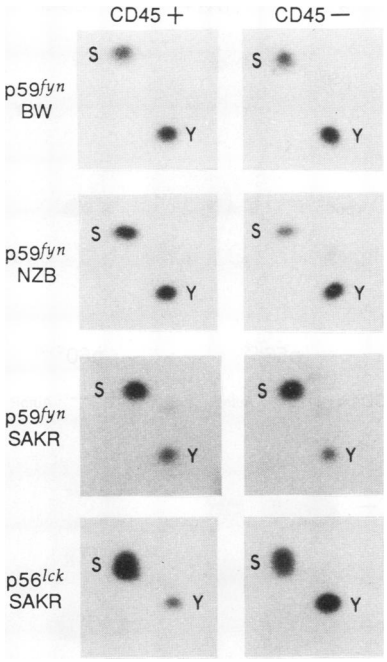

FIG. 1. Phosphoamino acid analysis of $p59^{fyn}$ and $p56^{lck}$. CD45-positive and -negative cells were labeled biosynthetically with $^{32}P_i$, and $p59^{fyn}$ and $p56^{lck}$ were isolated by immunoprecipitation and gel electrophoresis. The proteins were transferred to Immobilon, subjected to acid hydrolysis, and the resulting phosphoamino acids were analyzed by two-dimensional electrophoresis as described in Materials and Methods. The radioactive phosphoamino acids were detected by fluorography with an intensifying screen and prefogged film. S, phosphoserine; Y, phosphotyrosine.

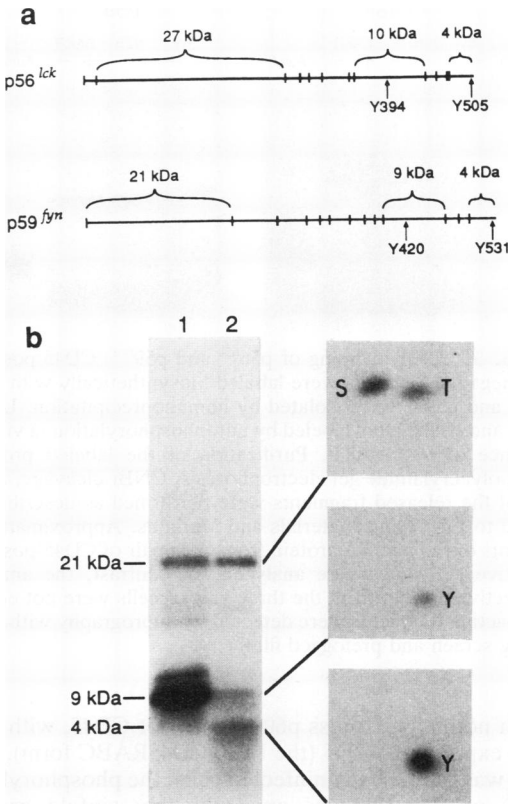

FIG. 2. CNBr mapping of $p56^{lck}$ and $p59^{fyn}$. (a) Schematic diagram of the major fragments generated by CNBr cleavage of $p56^{lck}$ and $p59^{fyn}$. (b) CNBr cleavage of $p59^{fyn}$ labeled in vitro (lane 1) or in vivo (lane 2) with $^{32}P_i$. $p59^{fyn}$ was labeled biosynthetically with $^{32}P_i$ or in vitro with [γ-^{32}P]ATP as described in Materials and Methods. The labeled proteins were purified by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and subjected to hydrolysis with CNBr. The released peptides were analyzed by SDS-polyacrylamide gel electrophoresis, and radioactive fragments were detected by fluorography with an intensifying screen and prefogged film. Phosphoamino acid analysis of the CNBr fragments of biosynthetically labeled $p59^{fyn}$ was performed as described in Materials and Methods. S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine.

by autophosphorylation. We infer therefore that the 4-kDa fragment does indeed contain the site of inhibitory phosphorylation, Tyr-531.

To compare the effect of the expression of CD45 on the phosphorylation of $p56^{lck}$ and $p59^{fyn}$, CD45-positive and -negative BW5147, NZB.1, and SAKRTLS12.1 cells were labeled biosynthetically with $^{32}P_i$, and $p56^{lck}$ and $p59^{fyn}$ were isolated by immunoprecipitation. The proteins were then hydrolyzed with CNBr, and the resulting fragments were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 3). The 27-kDa fragment of $p56^{lck}$ contains residues 15 to 260 and is phosphorylated predominantly on serine (20). The 9-kDa fragment of $p56^{lck}$ contains the site of autophosphorylation, Tyr-394 (20). The 4-kDa fragment contains the inhibitory site of phosphorylation, Tyr-505 (20).

As found previously by tryptic peptide mapping, the level of tyrosine phosphorylation of $p56^{lck}$ at Tyr-505 was increased noticeably in CD45-negative SAKRTLS12.1 cells (Fig. 3). To quantify this change, we analyzed the autoradiographic data by densitometry and then normalized the data such that the radioactivity in the 27-kDa fragment from each sample was equivalent. This approach uses the assumption that the phosphorylation of serines and threonines in the amino-terminal half of the protein are not affected by the expression of CD45. Although there is no evidence that the expression of CD45 affects the phosphorylation of these sites, we do not have proof that it does not. By using this means of quantification, the phosphorylation of Tyr-505 of $p56^{lck}$ was found to be increased approximately eightfold in the CD45-negative BW5147 cells, twofold in the CD45-negative NZB.1 cells, and sixfold in the CD45-negative SAKRTLS12.1 cells (Fig. 3).

Less pronounced differences in the phosphorylation of $p59^{fyn}$ were seen (Fig. 3). The apparent level of phosphorylation of the fragment containing the inhibitory site, Tyr-531, was increased approximately 2.5-fold in both CD45-negative BW5147 cells and CD45-negative SAKRTLS12.1 cells. There was no detectable change in the level of phosphorylation of this fragment in CD45-negative NZB.1 cells. A slight decrease in the phosphorylation of the fragment containing Tyr-420, the site of autophosphorylation, was apparent in the $p59^{fyn}$ from all of the CD45-negative cells. This is consistent with a slight decrease in the activity of the protein in the CD45-negative cells.

To determine whether expression of CD45 would affect the phosphorylation of $p60^{c\text{-}src}$, we generated cell lines that expressed both proteins. First, we infected CD45-positive and CD45-negative SAKRTLS12.1 cells, which express undetectable levels of $p60^{c\text{-}src}$, with a retrovirus expressing murine $p60^{c\text{-}src}$. Second, we infected rat 208F fibroblasts,

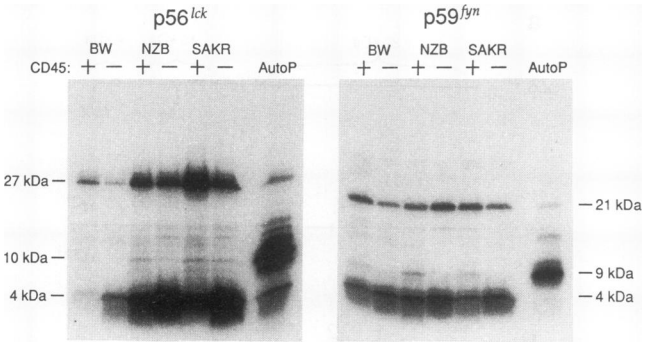

FIG. 3. CNBr mapping of p56^{lck} and p59^{fyn}. CD45-positive (+) and -negative (−) cells were labeled biosynthetically with $^{32}P_i$, and p56^{lck} and p59^{fyn} were isolated by immunoprecipitation. Unlabeled p56^{lck} and p59^{fyn} were labeled by autophosphorylation in vitro in the presence of [γ-^{32}P]ATP. Purification of the labeled proteins by SDS-polyacrylamide gel electrophoresis, CNBr cleavage, and analysis of the released fragments were performed as described in the legend to Fig. 2 and Materials and Methods. Approximately equal amounts of radioactive protein from each pair of CD45-positive and -negative cell lines were analyzed. In contrast, the amounts of radioactive protein from the three sets of cells were not equalized. Radioactive fragments were detected by fluorography with an intensifying screen and prefogged film.

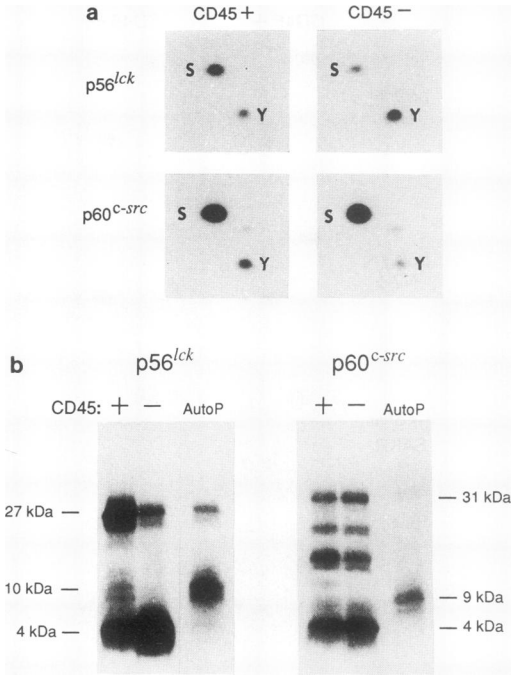

FIG. 4. Phosphoamino acid analysis and CNBr mapping of p56^{lck} and p60$^{c\text{-}src}$ CD45-positive (+) and -negative (−) SAKRTLS12.1 cells expressing murine c-*src* were labeled biosynthetically with $^{32}P_i$, and p56^{lck} and p60$^{c\text{-}src}$ were isolated by immunoprecipitation. Phosphoamino acid analysis was performed as described in the legend to Fig. 1 and in Materials and Methods, and CNBr cleavage was performed as described in the legend to Fig. 2 and in Materials and Methods. (a) Phosphoamino acid analysis. S, phosphoserine; Y, phosphotyrosine. (b) CNBr mapping.

which normally express p60$^{c\text{-}src}$ but not CD45, with a retrovirus expressing CD45 (the B220/CD45RABC form).

As was the case in uninfected cells, the phosphorylation of p56^{lck} on Tyr-505 was approximately sixfold greater in CD45-negative SAKRTLS12.1 cells expressing p60$^{c\text{-}src}$ than in CD45-positive SAKRTLS12.1 cells expressing p60$^{c\text{-}src}$ (Fig. 4). In contrast, the phosphorylation of p60$^{c\text{-}src}$ on tyrosine was essentially unchanged in the same pair of c-*src*-expressing CD45-positive and -negative SAKRTLS12.1 cells as gauged by phosphoamino acid analysis (Fig. 4a). CNBr cleavage of p60$^{c\text{-}src}$ yields a 31-kDa fragment containing the amino terminus of the protein, a 9-kDa fragment containing Tyr-416, the site of autophosphorylation, and a 4-kDa fragment containing Tyr-527, the regulatory site (4, 14). Labeling of the phosphorylated fragments of p60$^{c\text{-}src}$ from the CD45-positive and -negative cells was essentially identical (Fig. 4b). Scanning densitometry indicated that the phosphorylation of p60$^{c\text{-}src}$ at Tyr-527, present in the 4-kDa fragment, was increased by no more than 25% in the CD45-negative SAKRTLS12.1 cells.

Phosphorylation of endogenous p60$^{c\text{-}src}$ was unaltered in the rat 208F fibroblasts expressing CD45 (data not shown). This finding also suggests that p60$^{c\text{-}src}$ is a poor substrate of CD45. However, coexpression of CD45 and p56^{lck} in rat 208F fibroblasts does not lead to the dephosphorylation of p56^{lck} on tyrosine seen in lymphoid cells (data not shown). The lack of effect of CD45 on the phosphorylation of p60$^{c\text{-}src}$ in fibroblasts therefore may not be meaningful. The absence of an effect of CD45 on the phosphorylation of p56^{lck} in fibroblasts may imply that one or more proteins expressed only in lymphoid cells play a critical role in the interaction of CD45 and p56^{lck}.

DISCUSSION

We found previously that the loss of expression of the CD45 tyrosine protein phosphatase in murine SAKRTLS 12.1 cells increased the phosphorylation of p56^{lck} at Tyr-505, a site of inhibitory phosphorylation (28). We have shown here that a similar increase in phosphorylation of p56^{lck} occurs in two other CD45-negative murine T-cell lines. p59^{fyn} was found to undergo somewhat increased phosphorylation at a homologous site of inhibitory phosphorylation in two of these three CD45-negative cell lines. CD45 had, however, a much larger effect on the phosphorylation of p56^{lck} than on the phosphorylation of p59^{fyn} in all three of the cell lines examined here. These observations suggest strongly that CD45 is a natural regulator, either directly or indirectly, of these two *src*-like kinases and that its effect on *lck* is more pronounced than its effect on *fyn*. In contrast, CD45 had no effect on the phosphorylation of p60$^{c\text{-}src}$.

It is likely that the observed decrease in the phosphorylation of p56^{lck} at Tyr-505 in CD45-positive cells increases the activity of p56^{lck} in vivo, since mutation of Tyr-505 to Phe activates the protein biologically and enzymatically (2, 22). Catalytic activation of p56^{lck} by dephosphorylation of Tyr-505 is, however, much more difficult to demonstrate reproducibly by in vitro assays after isolation of the protein from cells (2, 28). We have not detected a decreased activity of p59^{fyn} isolated from CD45-negative cells by in vitro assays of the protein by using a variety of exogenous substrates. This is not wholly surprising. The difference in the phosphorylation of Tyr-531 in CD45-negative and -positive cells is small—no more than 2.5-fold—and even the fully dephosphorylated F531 mutant of p59^{fyn} exhibits at most 2.5-fold-greater activity than the wild-type protein when assayed in vitro (5). Nevertheless, the extent of autophosphorylation of the *fyn* kinase was decreased in all three CD45-negative cell

lines (Fig. 3). This is consistent with the idea that the protein exhibits slightly reduced activity in the CD45-negative cells.

CD45-negative T cells exhibit a greatly reduced responsiveness to stimulation through the T-cell receptor (18, 19, 30, 31, 39). Therefore, one or more substrates of CD45 must play a crucial role in T-cell activation. $p56^{lck}$ and possibly $p59^{fyn}$ may be these critical substrates of CD45. Stimulation through the T-cell receptor induces a rapid increase in tyrosine phosphorylation (10, 33) that is apparently essential for subsequent changes in the level of intracellular calcium and the activity of protein kinase C (15, 26). In some, but not all, CD45-negative cell lines, the ability of antibodies to the T-cell receptor to induce increased tyrosine phosphorylation is lost (18). The tyrosine phosphorylation stimulated by interaction with antigen and antigen-presenting cells or by cross-linking by anti-T-cell receptor antibodies may be catalyzed by either or both $p56^{lck}$ and $p59^{fyn}$ and could be abolished in CD45-negative cells by increased inhibitory phosphorylation of these protein kinases. Our finding that the expression of CD45 has a much more pronounced effect on the phosphorylation on $p56^{lck}$ than it does on $p59^{fyn}$ suggests that the inhibition of $p56^{lck}$ is more likely to be responsible for the reduced responsiveness of CD45-negative cells than is the inhibition of $p59^{fyn}$.

The possible greater importance of the hyperphosphorylation of $p56^{lck}$ in the unresponsiveness of CD45-negative T cells may have been anticipated by experiments examining the effects of mutants of $p56^{lck}$ and $p59^{fyn}$ on fibroblasts. The F505 mutant of *lck* readily transforms fibroblasts (2, 22). The homologous F531 mutant of *fyn* does not (5). Although results obtained with fibroblasts may not be relevant to an understanding of the regulation of these proteins in lymphoid cells, the lack of transforming activity of the mutant *fyn* protein in fibroblasts may indicate that simple dephosphorylation at Tyr-531 is not sufficient to render it fully active.

The fact that the expression of CD45 reduces the phosphorylation of $p56^{lck}$ and $p59^{fyn}$ at their regulatory sites is most simply explained by postulating that these two kinases are direct substrates of CD45. Indeed, it has been shown that CD45 can dephosphorylate both $p56^{lck}$ and $p59^{fyn}$ in vitro (27, 29). It is formally possible, however, that CD45 expression positively regulates a phosphatase that dephosphorylates the inhibitory site in these kinases or inhibits a protein kinase that phosphorylates the site in vivo. If CD45 is the tyrosine protein phosphatase responsible for the dephosphorylation of $p56^{lck}$ and $p59^{fyn}$, the question arises as to why it has a much more pronounced effect on $p56^{lck}$, a lesser effect on the closely related $p59^{fyn}$, and no effect on $p60^{c\text{-}src}$. Although this could reflect amino acid substrate specificity, the carboxy termini of $p56^{lck}$, $p59^{fyn}$, and $p60^{c\text{-}src}$ are extremely similar in sequence (7). More likely, the differences in the extent of dephosphorylation seen in vivo result from the catalytic domain of CD45 having greater access to $p56^{lck}$ than to $p59^{fyn}$ or $p60^{c\text{-}src}$ on the cytoplasmic face of the plasma membrane.

One interpretation of the finding that expression of CD45 is required for the response of lymphocytes to stimulation through the antigen receptor is that CD45 is activated by the binding of antigen. However, the fact that the tyrosine phosphorylation of both the *lck* and *fyn* kinases is affected by the expression of CD45 in the absence of antigenic stimulation suggests that CD45 exhibits constitutive phosphatase activity and maintains the *lck* and *fyn* kinases in an active form. In the case of $p56^{lck}$, approximately three-quarters of the population may normally not be phosphorylated at Tyr-505 in CD45-positive cells. Thus, CD45 need not necessarily undergo activation during stimulation through the antigen receptor. The increased tyrosine phosphorylation induced by antigenic stimulation could result in part from mobilization of preactivated protein kinases giving them increased access to specific polypeptide substrates.

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