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The Transmembrane Protein-tyrosine Phosphatase CD45 Is Associated with Decreased Insulin Receptor Signaling*

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Overexpression of the transmembrane protein-tyrosine phosphatase (PTPase) CD45 in nonhematopoietic cells results in decreased signaling through growth factor receptor tyrosine kinases. Consistent with these data, insulin receptor signaling is increased when the CD45-related PTPase LAR is reduced by antisense suppression in a rat hepatoma cell line. To test whether the hematopoietic cell-specific PTPase CD45 functions in a manner similar to LAR by negatively modulating insulin receptor signaling in hematopoietic cells, the insulin-responsive human multiple myeloma cell line U266 was isolated into two subpopulations that differed in CD45 expression. In CD45 nonexpressing (CD45−) cells, insulin receptor autophosphorylation was increased by 3-fold after insulin treatment when compared to CD45 expressing (CD45+) cells. This increase in receptor autophosphorylation was associated with similar increases in insulin-dependent tyrosine kinase activation. These receptor level effects were paralleled by postreceptor responses. Insulin-dependent tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and Shc was 3-fold greater in CD45− cells. In addition, insulin-dependent IRS-1/phosphatidylinositol 3-kinase association and MAP kinase activation in CD45− cells were also 3-fold larger. While expression of CD45 was associated with a decrease in the responsiveness of early insulin receptor signaling, interleukin 6-dependent activation of mitogen-activated protein kinase kinase and mitogen-activated protein kinase was equivalent between CD45− and CD45+ cells. These observations indicate that CD45 can function as a negative modulator of growth factor receptor tyrosine kinases in addition to its well-established role as an activator of *src* family tyrosine kinases.

The transmembrane protein-tyrosine phosphatase (PTPase)[1] CD45 has been shown to be an essential component of B-cell activation (reviewed in Ref. 1). Cell lines that lack CD45 are unable to respond efficiently to ligands that cross-link the B-cell antigen receptor. Responsiveness is restored, however, when CD45 is reconstituted. This work has recently been confirmed in CD45-deficient mice (2). Ironically, B-cell activation results in an increase in cellular tyrosine phosphorylation. The PTPase CD45 appears to function as a positive mediator of this increased cellular tyrosine phosphorylation by activating members of the *src* family protein-tyrosine kinases (1).

Although much of the work with CD45 has focused on its role as a positive component of B-cell antigen receptor signal transduction, recent work suggests that CD45 may also function as a negative modulator of tyrosine kinase signaling. For example, cross-linking studies have suggested that CD45 negatively modulates signaling through the Fcγ receptor on a monocytic cell line (3). More recently, the phosphorylated CD3 ζ chain has been shown to be a specific substrate of CD45, leading Furukawa *et al.* (4) to propose that CD45 is involved in the termination of T-cell activation. Furthermore, anti-CD45 monoclonal antibodies have been shown to inhibit interleukin 4 signal transduction (5).

Results demonstrating a negative role for CD45 are not surprising. We have shown that CD45 can blunt platelet-derived growth factor and insulin growth factor 1-dependent responses when transfected into the mouse mammary cell line C127 (6, 7). Similar results have been confirmed in 3T3L1 fibroblasts and the HepG2 hepatocellular carcinoma cell line,[2] as well as in human embryonic kidney fibroblast cells (8). We have recently shown that LAR, a transmembrane PTPase cloned on the basis of its homology to CD45 (9), can negatively modulate insulin receptor signal transduction within a rat hepatoma cell line (10). CD45 (also called leukocyte common antigen) and LAR (also called leukocyte common antigen-related) are high molecular weight transmembrane glycoproteins that contain two homologous tandem PTPase domains in their cytoplasmic regions. The extracellular regions for each of these PTPases do not possess any significant homology (9). Since CD45 has been well-characterized as an activator of intracellular tyrosine kinases, we wanted to investigate whether the structural elements it shares with LAR would allow it to function as a negative modulator of receptor tyrosine kinases. Since CD45 is expressed only by cells of hematopoietic origin, the human multiple myeloma cell line U266 was chosen for study because this cell line is known to respond to insulin (11–13).

The insulin receptor is a heterotetrameric protein complex composed of two extracellular α subunits and two transmembrane β subunits (reviewed in Ref. 14). After the α subunit binds insulin, the intrinsic tyrosine kinase activity of the β subunit increases, allowing it to phosphorylate itself as well as intracellular substrates. Two of these substrates include IRS-1

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[1] The abbreviations used are: PTPase, protein-tyrosine phosphatase; IRS-1, insulin receptor substrate 1; PI 3-kinase, phosphatidylinositol 3-kinase; MAP, mitogen-activated protein; MAPKK, MAP kinase kinase; GST, glutathione *S*-transferase; PAGE, polyacrylamide gel electrophoresis; IL-6, interleukin 6.

[2] B. A. Way and R. A. Mooney, unpublished observations.

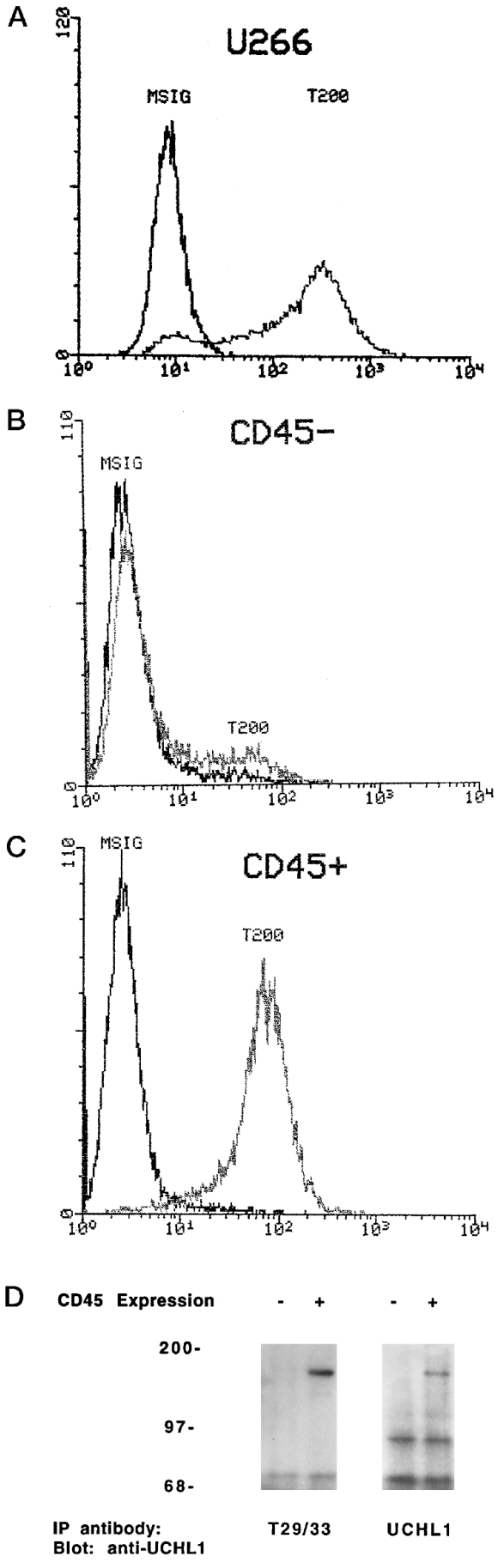

FIG. 1. **Isolation of CD45 nonexpressing (CD45−) and CD45 expressing (CD45+) subpopulations from the human multiple myeloma cell line U266.** Surface expression of CD45 was detected with the fluorescein isothiocyanate-labeled anti-CD45 antibody T200 (KC56). An isotype-matched irrelevant antibody, MSIG, was used as a control for nonspecific binding. *A*, surface expression of CD45 in U266 cells prior to cell sorting. *B* and *C*, surface expression of CD45 in U266 subpopulations isolated by fluorescence-activated cell sorting. *D*, Western blot analysis of CD45 expression in sorted U266 subpopulations. CD45 was immunoprecipitated from sorted cells with two different CD45 antibodies, T29/33 or UCHL-1, and blotted with UCHL-1. Identical results were obtained when whole cell lysates were blotted with UCHL-1 (data not shown).

and Shc. By examining the effect of CD45 on insulin receptor signal transduction, the role of CD45 as a negative modulator of growth factor receptor tyrosine kinase signaling was revealed.

EXPERIMENTAL PROCEDURES

Materials—Phosphotyrosine (4G10), IRS-1 (C-terminal), PI 3-kinase, Shc, *fyn*, and MAP kinase R2 (*erk*1-CT) antibodies were purchased from Upstate Biotechnology, Inc. (UBI, Saranac Lake, NY). GST-MAP kinase was purchased from UBI. Fluorescein isothiocyanate-conjugated CD45 antibody T200 (KC56) was from Coulter. Other CD45 antibodies were: HLe-1 (Becton Dickinson), T29/33 (Boehringer Mannheim), and UCHL-1 (Dako, Carpinteria, CA). The insulin receptor antibody was a generous gift from Dr. Richard Furlanetto (University of Rochester). RPMI cell culture media and fetal bovine serum were from Life Technologies, Inc. [γ-^{32}P]ATP (3000 Ci/mmol) and enhanced chemiluminescence detection reagents were purchased from Amersham.

Cell Culture—The human multiple myeloma cell line U266 (15) was cultured in a humidified atmosphere of 5% CO_2, 95% air at 37 °C in RPMI 1640 media supplemented with 10% fetal bovine serum. Cells were split 1:1 every 3 days. Prior to experiments, cells were suspended in serum-free media supplemented with 1% bovine serum albumin for 18 h. Cell counts were performed on an S Plus Stacker Jr. (Coulter).

Isolation of CD45− and CD45+ U266 Subpopulations—The U266 cell line was immunolabeled with the fluorescein isothiocyanate-conjugated CD45 antibody T200 (KC56). CD45− and CD45+ cells were isolated by fluorescence-activated cell sorting as described previously (6). One cycle of fluorescence-activated cell sorting yielded >99% pure CD45+ cells. Two cycles were needed to isolate >95% pure CD45− cells. Differential expression of CD45 in the subpopulations was maintained for more than 2 months. CD45 protein levels in the CD45− and CD45+ subpopulations were determined by Western blot analysis. 15×10^6 cells were solubilized in 50 mM Tris, pH 7.4, 1% Triton X-100, 100 mM NaCl, 25 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride. After diluting the Triton X-100 concentration to 0.1%, CD45 was immunoprecipitated with either T29/33 or UCHL-1. UCHL-1 was used as the blotting antibody, using methods described previously (7).

Intact Cell Tyrosine Phosphorylation—Intact cell tyrosine phosphorylation was measured as described previously (7). Briefly, suspensions of 25×10^6 CD45− and CD45+ cells were incubated with insulin at the indicated concentrations for 5 min and harvested in 1.0 ml of lysis buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml benzamidine, 1 mM orthovanadate, 50 mM NaF, and 10 mM tetrasodium pyrophosphate). Tyrosine-phosphorylated proteins were isolated by immunoprecipitation with the indicated antibody, separated by SDS-PAGE, and transferred to polyvinylidene difluoride. Immunoblots were visualized by ^{125}I-conjugated goat anti-rabbit (in conjunction with an unlabeled rabbit anti-mouse bridging layer) as described (7). Where indicated, immunoblots were visualized by enhanced chemiluminescence (ECL™) as described (16). Densitometry was performed with an XRS image scanner and Bio Image Software.

MAP Kinase and MAP Kinase Kinase Activity—5×10^6 CD45− and CD45+ cells were treated with either insulin or IL-6 at the indicated concentrations for 5 min. Cells were harvested in lysis buffer supplemented with 1 mM dithiothreitol and 1 mM EGTA. For the MAP kinase kinase assay, an equal volume of total cell lysate was mixed with a reaction mixture containing 100 mM Tris-HCl, pH 7.4, 4 mM EGTA, 2 mM dithiothreitol, 20 mM $MgCl_2$, and 0.1 μCi/μl tracer [γ-^{32}P]ATP. 1 μg of GST-MAP kinase (UBI) was then added to the mixture and incubated for 30 min. The reaction was stopped by 10-fold dilution with lysis buffer containing 1 mM ATP. After washing the agarose beads 3 times with lysis buffer, SDS-PAGE sample buffer was added. The samples were boiled for 5 min and separated on an SDS-PAGE gel. Phosphorylated GST-MAP kinase was detected by autoradiography.

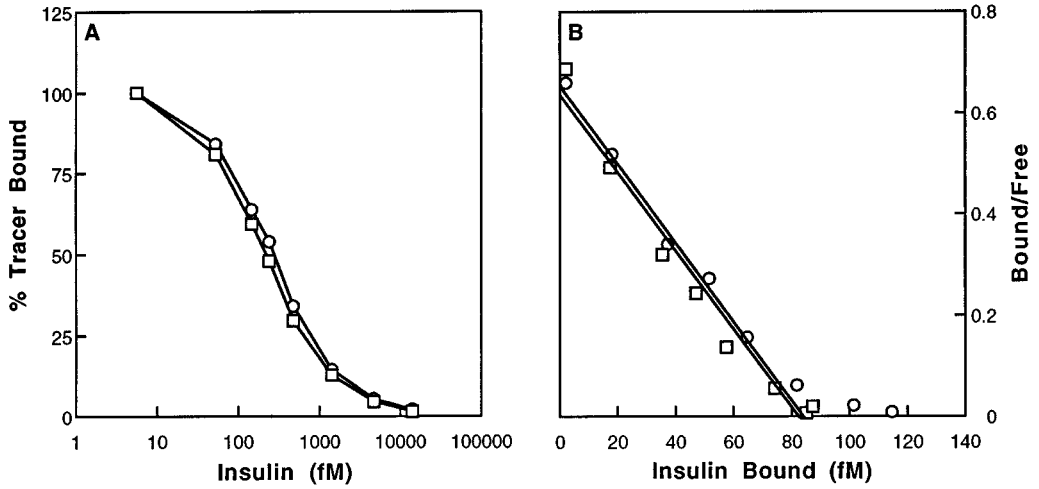

FIG. 2. **Analysis of insulin receptor expression on CD45− and CD45+ U266 cells.** *A*, competitive insulin binding. CD45− and CD45+ U266 cells were incubated for 3 h at 15 °C with 11.1 pM ^{125}I-conjugated insulin (2200 Ci/mmol) plus the insulin concentrations indicated, as described previously (11). Cells were washed, and cell-associated radiolabel was quantitated. Plots are an average of two experiments. *B*, Scatchard analysis of data in *A*. *Open squares*, CD45− cells. *Open circles*, CD45+ cells.

The MAP kinase assay was performed similarly. MAP kinase was immunoprecipitated from growth factor-treated cells with a MAP kinase antibody (UBI). To the immunoprecipitate was added a reaction buffer containing 50 mM Tris-HCl, pH 7.4, 2 mM EGTA, 1 mM dithiothreitol, 10 mM $MgCl_2$, 1 mg/ml myelin basic protein, 0.1 mg/ml protein kinase inhibitor (Sigma), and 2 μM ATP containing 0.01 μCi/μl [γ-^{32}P]ATP. After a 5-min incubation at room temperature, the reaction was terminated by adding SDS-PAGE sample buffer and boiling for 5 min. The phosphorylated proteins were separated on an SDS-PAGE gel and visualized by autoradiography.

fyn Kinase Assay—5 × 10^6 CD45− and CD45+ cells were harvested in lysis buffer and *fyn* immunoprecipitates were obtained. The *fyn* kinase assay was performed exactly as described previously (17). In brief, immune complexes were washed twice in RIPA buffer, once in high salt buffer (10 mM Tris, pH 7.2, 150 mM NaCl), and twice in kinase assay buffer (10 mM Tris, pH 7.2, 5 mM $MnCl_2$), and resuspended in 50 μl of kinase assay buffer, containing 5 μM [γ-^{32}P]ATP (20 μCi/nmol) and 10 μg of acid and heat-denatured rabbit muscle enolase. After 10 min, 50 μl of 2 × SDS-PAGE sample buffer was added. The samples were boiled for 5 min and separated on an SDS-PAGE gel. Phosphorylated enolase was detected by autoradiography.

RESULTS

Flow cytometric analysis of the human multiple myeloma U266 cell line revealed a heterogeneous expression of CD45, ranging from nonexpressers to high expressers (Fig. 1*A*). Two stable U266 subpopulations, CD45− and CD45+ (Fig. 1, *B* and *C*), were isolated with fluorescence-activated cell sorting. This technique has been used successfully by other investigators to isolate CD45− and CD45+ subpopulations (18). After two cycles of sorting, CD45− and CD45+ cells were >95% and >99% pure, respectively. The absence of CD45 expression within CD45− cells was confirmed by Western blot analysis (Fig. 1*D*).

The first parameter examined was insulin receptor binding and affinity. As shown by Fig. 2, CD45− and CD45+ cells possessed equivalent numbers of insulin receptors with equivalent binding affinity. In addition, each of these subpopulations possessed equivalent cell growth rates, morphology, immunoglobulin synthesis, and total protein per cell (data not shown).

If CD45 negatively modulated insulin receptor signal transduction at the level of the receptor, then receptor activation, particularly autophosphorylation (14), should be increased in CD45− cells. As shown by Fig. 3*A*, insulin-dependent receptor autophosphorylation was increased by 3.2 ± 0.3-fold (mean ± 1/2 range) and 3.5 ± 0.2-fold (mean ± 1/2 range) in CD45− cells as compared to CD45+ cells at 2 nM and 200 nM insulin treatment, respectively. This increase in insulin receptor autophosphorylation in CD45− cells supports the hypothesis that CD45

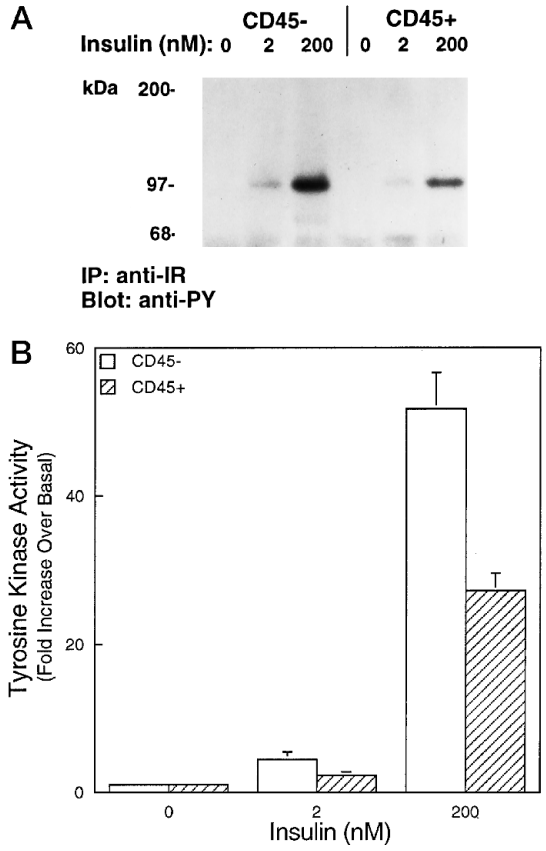

FIG. 3. **Effect of CD45 expression on insulin receptor autophosphorylation and tyrosine kinase activation.** *A*, intact cell receptor autophosphorylation. After incubating 25 × 10^6 cells for 5 min with insulin at the indicated concentrations, whole cell lysates were immunoprecipitated with an insulin receptor antibody, separated by SDS-PAGE, and electro-transferred to polyvinylidene difluoride paper. Protein bands were located by autoradiography after incubation with 4G10. *B*, intact cell tyrosine kinase activation. After incubating 5 × 10^6 cells for 5 min with insulin at the indicated concentrations, whole cell lysates were immunoprecipitated with an insulin receptor antibody. Immunoprecipitated receptors were then subjected to a tyrosine kinase assay using poly(Glu-Tyr)(4:1) as an exogenous substrate. Data represent mean ± S.E. from three independent experiments performed in duplicate. *Open bars* represent CD45− cells, and *hatched bars* represent CD45+ cells.

can function as a negative modulator of insulin receptor activation in hematopoietic cells. Since increased receptor autophosphorylation may not necessarily correlate with increased receptor tyrosine kinase activation, receptor kinase activity was specifically examined.

After insulin treatment, insulin receptors were immunoprecipitated in the presence of phosphatase inhibitors and subjected to a tyrosine kinase assay with poly(Glu-Tyr)(4:1). We have previously shown that this technique preserves insulin-dependent increases in receptor tyrosine kinase activity (10). As shown by Fig. 3*B*, insulin treatment led to a dose-dependent increase in receptor tyrosine kinase activity. The activity seen in CD45− cells was increased by approximately 2-fold at each insulin concentration tested when compared to CD45+ cells. The fold increase over basal in tyrosine kinase activation was 4.4 ± 1.0 (mean ± S.E.) at 2 nM insulin and 51.8 ± 4.8 (mean ± S.E.) at 200 nM insulin in CD45− cells. In CD45+ cells, the fold increase over basal in tyrosine kinase activation was 2.2 ± 0.4 (mean ± S.E.) at 2 nM insulin and 27.2 ± 2.4 (mean ± S.E.) at 200 nM insulin. Insulin receptor basal activity between CD45− and CD45+ cells was indistinguishable. As an additional control, insulin receptors were immunoprecipitated from untreated cells. *In vitro* insulin treatment of these receptors resulted in a dose-dependent increase in receptor tyrosine kinase activation. Under these conditions, however, the fold increase over basal was equivalent between CD45− and CD45+ cells (data not shown). These data indicate that the intact cell differences in insulin receptor signaling between CD45− and CD45+ cells were not due to events occurring during or after cell lysis.

The best characterized substrate of the insulin receptor is the insulin receptor substrate 1 (IRS-1, recently reviewed in Refs. 19 and 20). After insulin treatment, this protein becomes tyrosine-phosphorylated and associates with phosphatidylinositol 3-kinase (PI 3-kinase). As shown in Fig. 4*A*, insulin treatment of U266 cells resulted in an increase in IRS-1 tyrosine phosphorylation. This increase, however, was amplified by 3.1 ± 0.3-fold (mean ± 1/2 range) and 3.3 ± 0.2-fold (mean ± 1/2 range) in CD45− cells when compared to CD45+ cells at 2 nM and 200 nM insulin, respectively. Likewise, insulin-dependent association of PI 3-kinase with IRS-1 was increased 3-fold in CD45− cells when compared to CD45+ cells (Fig. 4*B*). Previously, we have documented the absence of a marked dose dependence in insulin-dependent PI 3-kinase activation in the U266 cell line (12).

If CD45 was affecting insulin receptor signal transduction at the level of the insulin receptor, then all kinase-dependent events should be affected to a similar degree by differing CD45 levels. To demonstrate that differing CD45 levels weren't affecting IRS-1-mediated events specifically, insulin-dependent Shc tyrosine phosphorylation was examined. As shown in Fig. 4*C*, insulin-dependent Shc tyrosine phosphorylation was increased by 3-fold in CD45− cells when compared to CD45+ cells at each insulin concentration tested.

Activation of the MAP kinase pathway is a well-characterized post-receptor response to insulin treatment (21). Since recent studies have indicated that MAP kinase activation is also increased by IL-6 treatment (22, 23), examination of this signaling pathway provided an opportunity to test the specificity of CD45 action. As shown in Fig. 5*A*, insulin and IL-6 treatment resulted in an increase in MAP kinase activation. Consistent with an effect of CD45 on the insulin receptor, insulin-dependent MAP kinase activation was increased 3-fold in CD45− cells when compared to CD45+ cells. IL-6-dependent activation of MAP kinase, however, was equivalent between CD45− and CD45+ cells.

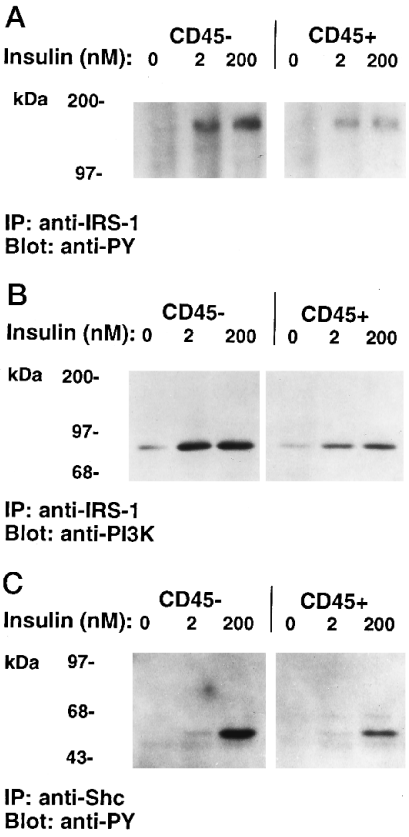

FIG. 4. **Effect of CD45 expression on insulin-dependent substrate tyrosine phosphorylation and IRS-1/PI 3-kinase complexing.** After incubating 25×10^6 U266 cells for 5 min with insulin at the indicated concentrations, whole cell lysates were immunoprecipitated with an IRS-1 (C-terminal) or a Shc antibody (UBI). The immunoprecipitated proteins were separated by SDS-PAGE and transferred onto polyvinylidene difluoride paper. Protein bands were visualized by ECL™ after being blotted with either a phosphotyrosine or a PI 3-kinase antibody. *A*, Western blot analysis of IRS-1 tyrosine phosphorylation. *B*, Western blot analysis of PI 3-kinase/IRS-1 association. *C*, Western blot analysis of Shc tyrosine phosphorylation.

To demonstrate that CD45 was altering MAP kinase activation through a pathway that involves MAP kinase kinase (MAPKK) (23), MAPKK was specifically examined. When ligand-dependent MAPKK activation was examined, results similar to those noted for MAP kinase activation were observed. Insulin-dependent changes in MAPKK activation were 3-fold greater in CD45− cells when compared to CD45+ cells. Under identical conditions, however, IL-6-dependent increases in MAPKK activation were again equivalent between CD45− and CD45+ cells (Fig. 5*B*).

Since CD45 has been studied primarily as an activator of *src* family kinases (1), the basal activity of *fyn*, a *src* family kinase, was examined in CD45+ and CD45− populations. Kinase activity in *fyn* immunoprecipitates, as measured by phosphorylation of the substrate enolase, was severalfold lower in CD45− cells when compared to CD45+ cells (Fig. 6). *fyn* activity was unaltered by insulin. Autophosphorylation of *fyn* was below the level of detection in the U266 cell line.

DISCUSSION

The variable expression of the transmembrane PTPase CD45 on U266 cells permitted the isolation of two stable subpopulations: CD45 non-expressers (CD45−) and CD45 expressers (CD45+). Each insulin-dependent response examined, receptor autophosphorylation, receptor tyrosine kinase activation, IRS-1 tyrosine phosphorylation, IRS-1/PI 3-kinase complexing, Shc tyrosine phosphorylation, MAP kinase kinase activation,

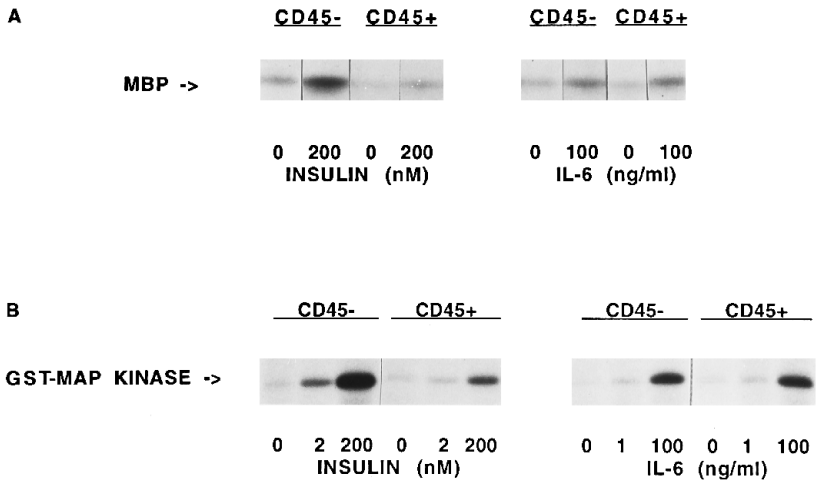

FIG. 5. **Effect of CD45 expression on insulin- and IL-6-dependent MAP kinase and MAP kinase kinase activation.** 5×10^6 CD45− and CD45+ cells were treated with the indicated concentrations of insulin or IL-6 for 5 min. After solubilization, lysates were subjected to either a MAP kinase or a MAP kinase kinase assay as described under "Experimental Procedures." These assays were linear with time and substrate concentration (data not shown). *A*, autoradiogram of the phosphorylated products of the MAP kinase assay. *MBP*, myelin basic protein. *B*, autoradiogram of phosphorylated GST-MAP kinase by MAP kinase kinase. Under the conditions of this assay, GST is not phosphorylated (data not shown).

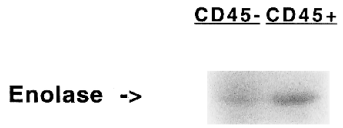

FIG. 6. **Activity of the *src* family kinase, *fyn*, in CD45− and CD45+ cells.** *fyn* was immunoprecipitated from cell lysates and added to a kinase assay containing acid- and heat-denatured rabbit muscle enolase. After 10 min, the reactions were terminated and proteins were separated by SDS-PAGE. Phosphorylated enolase was visualized by autoradiography.

and MAP kinase activation, was increased by 2- to 3-fold in CD45− cells when compared to CD45+ cells. Despite these ligand-dependent differences, basal activity of the insulin receptor was equivalent between CD45− and CD45+ cells. Most significantly, the number and binding affinity of insulin receptors was equivalent between CD45− and CD45+ cells. Interestingly, basal activity of the *src* family kinase member *fyn* was decreased severalfold in CD45− cells. This is consistent with evidence indicating that inhibitory tyrosine residues on *lck* and *fyn* are hyperphosphorylated in CD45-deficient cell lines (24). In addition, interleukin 6 (IL-6)-dependent activation of MAP kinase kinase and MAP kinase was equivalent between CD45− and CD45+ cells. These results demonstrate that CD45 possesses functions not limited to the activation of *src* family tyrosine kinases.

Although hematopoietic cells classically have not been defined as an insulin-responsive tissue, recent work has demonstrated many examples of insulin receptor signaling within these cells. Insulin responses have been reported in human IM-9 and J558L (mouse plasmacytoma) (13), myeloid (25), multiple myeloma (11–13), and lymphoblastoid (26) cell lines. Recent work with the 32D cell line, which has a myeloid progenitor phenotype, has indicated that insulin can be a potent mitogenic factor for hematopoietic cells that possess intact insulin signaling pathways (27). As a role for insulin in hematopoietic cell signal transduction evolves, the function of CD45 as a physiological modulator of insulin receptor signaling may well become more important. An important extension of the current work would be to determine whether the impact of CD45 on insulin receptor signaling is preserved in hematopoietic cell lines other than the U266 cell line.

Whether CD45 directly or indirectly affects the insulin receptor remains to be determined. Since the CD45− and CD45+ cell populations were isolated by fluorescence-activated cell sorting and not by a specific gene knock-out technique, we cannot formally exclude the possibility that the enhancement of insulin receptor signaling within CD45− cells may be due to an unknown factor. It is possible, for example, that CD45 functions by activating another PTPase. This would be consistent with its well-established role as a positive signal transducer. The actual site of CD45 action can provide important information regarding the mechanism by which transmembrane PTPases can modulate receptor tyrosine kinase signaling. Since decreased CD45 expression resulted in severalfold increases in insulin-dependent receptor autophosphorylation and receptor kinase activation, it appears that CD45 functions at the level of the insulin receptor. This conclusion is supported by the IRS-1 and Shc tyrosine phosphorylation data. Insulin-dependent IRS-1 and Shc tyrosine phosphorylation were increased by a similar 3-fold in CD45− cells when compared to CD45+ cells at each insulin concentration tested. Furthermore, the lack of a change in IL-6 responses between CD45− and CD45+ cells supports a receptor level site of action since insulin and IL-6 activate MAP kinase through similar pathways. The possibility that CD45 also functions to a minor degree at other post-receptor sites, such as IRS-1 or Shc, cannot be excluded.

We have been interested specifically in the transmembrane class of PTPases as physiological regulators of receptor tyrosine kinase signaling due to their localization within the same subcellular compartment. We have shown that the widely expressed transmembrane PTPase LAR can function as a negative modulator of growth factor receptor signaling within a hepatoma cell line (10). Here, we were interested whether the hematopoietic cell-specific transmembrane PTPase CD45 could perform a similar function within a hematopoietic cell line. CD45 and LAR are high molecular weight, glycosylated, transmembrane PTPases that possess two highly homologous tandem PTPase domains in their cytoplasmic regions. The membrane-proximal PTPase domains for LAR and CD45 possess catalytic activity, whereas the membrane distal PTPase domains have been suggested to serve an important role in substrate specificity. Here, we show that CD45 possesses functional as well as structural similarities to LAR. Reduction of either one of these PTPases can increase insulin receptor signaling. Interestingly, a 63% reduction in LAR resulted in a

3–4-fold increase in insulin receptor signaling. A complete absence of CD45, however, was associated with only a 2–3-fold increase in insulin receptor signaling. It is possible that CD45 and LAR differ in their substrate specificities. There is some evidence indicating that CD45 and LAR differ with regard to substrate interactions. Furukawa *et al.* (4) have shown that the phosphorylated CD3 ζ chain is a specific and high affinity substrate for CD45; LAR, however, does not bind this protein. LAR might have a higher selectivity for inactivating the insulin receptor than CD45. This would be compatible with evidence indicating that LAR is a major PTPase expressed by the classic insulin-responsive tissues fat, muscle, and liver. CD45, of course, isn't expressed by these tissues.

In addition, it is probable that U266 cells possess PTPases other than CD45 that are capable of regulating insulin receptor signaling. A PTPase presumably exists within CD45− cells that can terminate insulin receptor signaling. An examination of PTPases expressed in the pre-B-cell line NALM-6 (28), for example, revealed that this hematopoietic cell line expresses at least seven different PTPases. One or more of these PTPases might be modulating insulin receptor signaling within U266 cells. The possibility that LAR is performing this function is currently being pursued.

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