Differential Function of PTPα and PTPα Y789F in T Cells and Regulation of PTPα Phosphorylation at Tyr-789 by CD45*

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CD45 is a major membrane protein tyrosine phosphatase (PTP) expressed in T cells where it regulates the activity of Lck, a Src family kinase important for T cell receptor-mediated activation. PTPα is a more widely expressed transmembrane PTP that has been shown to regulate the Src family kinases, Src and Fyn, and is also present in T cells. Here, PTPα was phosphorylated at Tyr-789 in CD45− T cells but not in CD45+ T cells suggesting that CD45 could regulate the phosphorylation of PTPα at this site. Furthermore, CD45 could directly dephosphorylate PTPα in vitro. Expression of PTPα and PTPα Y789F in T cells revealed that the mutant had a reduced ability to decrease Fyn and Cbp phosphorylation, to regulate the kinase activity of Fyn, and to restore T cell receptor-induced signaling events when compared with PTPα. Conversely, this mutant had an increased ability to prevent Pyk2 phosphorylation and CD44-mediated cell spreading when compared with PTPα. These data demonstrate distinct activities of PTPα and PTPα Y789F in T cells and identify CD45 as a regulator of PTPα phosphorylation at tyrosine 789 in T cells.

Protein tyrosine phosphatases (PTPs), together with protein tyrosine kinases, are responsible for controlling the level of tyrosine phosphorylation within cells. In general, basal tyrosine phosphorylation levels in unstimulated cells are relatively low, suggesting that protein tyrosine phosphatase activity prevails over tyrosine kinase activities under resting conditions. Stimulation of cells via a variety of receptors linked to tyrosine kinases can induce tyrosine phosphorylation cascades that lead to cell activation or modulation of cellular function. In T lymphocytes, CD45 is a major protein tyrosine phosphatase expressed at the T cell membrane where it is thought to contribute over 90% of the membrane-associated PTP activity (1). Recognition of antigen by the T cell receptor (TCR) results in induction of a tyrosine phosphorylation cascade leading to T cell activation and is driven primarily by the activity of the Src family kinase (SFK), Lck (2). Counter-intuitively, CD45 is required for the efficient progression of this TCR-induced tyrosine phosphorylation signal, as it is required to dephosphorylate the negative regulatory site (Tyr-505) of Lck (reviewed in Refs. 3–5). This relieves inhibitory constraints on Lck and primes it for subsequent autophosphorylation at tyrosine 394 and activation. This activating function of CD45 and its requirement for efficient TCR signaling are well established. However, evidence from CD45−/− thymocytes and certain CD45− T cell lines indicates that the absence of CD45 can result in enhanced Lck phosphorylation at both the negative regulatory and autophosphorylation sites, suggesting a role for CD45 in both the activation and inactivation of Lck (4, 6, and reviewed in Ref. 7). Thus the function of CD45 may be to keep Lck under control, allowing activation, but preventing sustained activation or inactivation. It has been difficult to examine the role of CD45 in inactivating Lck and down-regulating TCR signaling because CD45 is also needed for activation. However, antibody cross-linking of CD4 and CD44 can result in Lck activation independently of CD45, thereby allowing observation of the inactivating role of CD45 and its dephosphorylation of Tyr-394 on Lck (8–10). CD45 has also been reported to affect the phosphorylation state of Fyn in T cells (11); although this effect has always been less pronounced than for Lck. Likewise, although Fyn has been implicated in TCR signaling events, its effect on TCR signaling events is less dramatic than that of Lck (12).

SFKs are widely expressed, whereas CD45 expression is restricted to leukocytes, indicating that other PTPs must regulate these enzymes in other cell types. PTPα has been shown to dephosphorylate the negative regulatory site of Src and Fyn in other cell types, whereas cytosolic PTPs such as SHP-1, 2, PEP, and PTP1B have been implicated in dephosphorylation at the autophosphorylation site (13 and reviewed in Ref. 14). Some of these PTPs are also present in T cells where they have been shown to modulate SFK phosphorylation, indicating that regulation of SFKs can be controlled by multiple phosphatases. Although expression of PTPα in a CD45− T cell line did not substantially affect Lck or Fyn phosphorylation, it did partially restore CD3-mediated TCR signaling events, suggesting that it can partially substitute for some CD45 activities (15). A comparison of CD45 and PTPα activities revealed that PTPα was a much less active PTP under the in vitro conditions tested. Furthermore, analysis of thymocyte development and T cell acti-
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...immunoprecipitated from CD45 and CD45 mouse T lymphoma cells (CD45+ and CD45− T cells) expressing TCR/CD3 and PTPα were described previously (15). VSVG-tagged forms of PTPα (18) and PTPα-Y789F were transfected into CD45+ and CD45− T cells and sorted for high expression. Cells were maintained in Dulbecco’s modified Eagle’s medium with 10% (v/v) horse serum supplemented with 100 μg/ml streptomycin-penicillin, 3 mM histidinol (Sigma), and 1 mg/ml G418 (Invitrogen) to maintain the expression of TCR/CD3 and PTPα, respectively. Thymocytes and splenocytes were isolated from C57Bl/6 mice and CD45+/− mice (19).

Reagents and Antibodies—Purified recombinant His6-tagged murine CD45 cytoplasmic domain proteins were described previously (20, 21). Anti-PTPα rabbit antisera (22), anti-Lck (BD Transduction Laboratories or R54-3B, 23), anti-CD45 phospho-Tyr-529 Fyn (Biosource International), phospho-Zap-70, phospho-Zap-70 (Tyr-319) (Cell Signaling) Pyk2, Cbp, Lck, and Pyk2 were identified by immunoblotting described (16). Anti-PTPα rabbit antisera (22), anti-Lck (BD Transduction Laboratories or R54-3B, 23), anti-CD45 phospho-Tyr-529 Fyn (Biosource International), and anti-Cbp antisera (24) were used. Protein A-conjugated HRP (Bio-Rad) and goat anti-mouse IgG HRP (Southern Biotechnology) were used in Western blots.

Immunoprecipitation and Immunoblotting—Cells were lysed in either 1% Triton X-100 with 150 mM NaCl or KCl, 20 mM Tris-HCl (pH 7.5), 2 mM EDTA or radioimmune precipitation assay buffer, with 0.5 mM sodium orthovanadate, 0.2 mM sodium molybdate, 0.2 mM phenylmethanesulfonfluoride, 1 mg/ml leupeptin, 1 mg/ml aprotinin, and 1 mg/ml peptatin. Cell lysates were incubated on ice for 20 min and then centrifuged at 14,000 × g for 10 min at 4 °C. PTPα, Fyn, Lck, Cbp, or Pyk2 was immunoprecipitated from CD45+ and CD45− T cells, thymocytes, or splenocytes, essentially as described previously (9, 15, 16). Samples were analyzed on 10% acrylamide gels by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane (Immobilon P, Millipore). PTPα, Fyn, p529 Fyn, Cbp, Lck, and Pyk2 were identified by immunoblotting with the respective antibodies and the overall tyrosine phosphorylation state identified by 4G10 mAb followed by protein A HRP or anti-mouse IgG-HRP. Blots were developed using ECL or ECL plus (Amersham Biosciences) and exposed to BioMax Film from Eastman Kodak Co.

In Vitro Dephosphorylation Assay—PTPα and Lck were immunoprecipitated from CD45− T cells transfected with PTPα as described above. Immune complexes bound to beads were washed three times with cold PTP buffer (50 mM Tris, pH 7.2, 1 mM EDTA, 0.1% β-mercaptoethanol) and then incubated with 400 ng (20 μl) of purified recombinant His6-CD45 or His6-CD45C817S proteins at 30 °C from 0 to 10 min. The reactions were stopped by immersion in a dry ice/ethanol bath, and addition of 10 μl of 3X reducing sample buffer. Samples were electrophoresed on a 10% SDS-PAGE and transferred to PVDF. The amount of phosphorylated PTPα or Lck remaining was detected by 4G10 (1:5000) and goat anti-mouse-HRP or protein A-HRP. The tyrosine-phosphorylated bands were analyzed by densitometric analysis using Alphaimager™ software. The amount of tyrosine phosphorylation per unit PTPα at time 0 was taken as 100%.

Kinase Assay—Fyn was immunoprecipitated from 300 to 400 μg of protein lysate. The kinase activity of these immunoprecipitates was determined in 20 μl of kinase buffer containing 10 mM PIPES, pH 7, 5 mM MnCl2, 0.5 mM dithiothreitol, and 10 μCi [γ-32P]ATP, and with or without 0.2 unit of enolase (Sigma) at 30 °C for 10 min (18). The enolase was treated with 10-mM sodium acetate, pH 3.5, at 37 °C for 5 min prior adding to the kinase buffer. Reactions were terminated by adding 20 μl of 2 × SDS sample buffer, boiled for 5 min, and subjected to SDS-PAGE. The SDS-PAGE gels were dried and autoradiographed. Another set of immunoprecipitates was immunoblotted with anti-Fyn antibodies to determine the amount of immunoprecipitated protein (data not shown).

TCR/CD3 Stimulation—This was performed essentially as described (16).

Cell Spreading Assay—Untransfected and PTPα transfected CD45− T cells were immobilized on anti-CD44 mAb, essentially as described (9). After 2 h of incubation at 37 °C, the cells were fixed with 4% paraformaldehyde and the cell length measured. The two-tailed t test was used to determine statistical significance.

RESULTS

PTPα Is Tyrosine Phosphorylated in T Cells Lacking CD45—Transfection of PTPα into CD45+ and CD45− BW5147 T lymphoma cells (hereafter referred to as CD45+ and CD45− T cells, respectively) and selection of PTPα positive clones revealed that PTPα was tyrosine phosphorylated in CD45− T cells, but not in CD45+ T cells (Fig. 1A). Because PTPα is constitutively phosphorylated at tyrosine 789 in NIH 3T3 fibroblasts (25), PTPα-Y789F was expressed in CD45+ and CD45− T cells. This mutated form of PTPα was not tyrosine phosphorylated in CD45− T cells, but not in CD45+ T cells (Fig. 1A). Therefore, PTPα is constitutively phosphorylated at tyrosine 789 in CD45− T cells, but not in CD45+ T cells (Fig. 1A). Inhibiting that PTPα was phosphorylated at tyrosine 789 in CD45− T cells. It also suggested that the presence of CD45 facilitated dephosphorylation of PTPα at Tyr-789. To determine whether CD45 was able to regulate the phosphorylation state of endogenous PTPα in T cells, the phosphorylation state of PTPα was examined in thymocytes and splenocytes from CD45+ (CD45+/+, C57Bl/6), and CD45 null (CD45−/−) mice (19). Fig. 1B shows that the phosphorylation of endogenous PTPα in both thymocytes and splenocytes isolated from CD45−/− mice was significantly higher than from CD45+/+ cells. These data strongly suggest an unexpected role for CD45 in regulating PTPα phosphorylation in T cells.
CD45 Can Directly Dephosphorylate Tyrosine 789 of PTPα in Vitro—To determine whether CD45 was able to directly dephosphorylate PTPα at tyrosine 789, dephosphorylation of phosphorylated PTPα was examined in vitro. Purified active recombinant CD45 cytoplasmic domain (αCD45) or mutated inactive rCD45cytC817S (iCD45; 20) were used to dephosphorylate PTPα immunoprecipitated from CD45− T cells. This was compared with immunoprecipitated Lck, a well established substrate of CD45 in T cells. Active, recombinant CD45 (a CD45) dephosphorylated PTPα at a rate similar to that of Lck (Fig. 2, A and B). Importantly, no significant dephosphorylation was observed in the presence of inactive CD45, indicating that PTPα itself was not making a substantial contribution to its own dephosphorylation. This is also consistent with the inability of PTPα to dephosphorylate itself in CD45− T cells. These in vitro data indicate that CD45 can efficiently and directly dephosphorylate PTPα and raise the possibility that CD45 may directly dephosphorylate PTPα at tyrosine 789 in T cells.

Tyrosine Phosphorylation of PTPα Is Significantly Reduced by the SFK Inhibitor, PP2—Src has been implicated in the phosphorylation of tyrosine 789 of PTPα (25, 26). As CD45 can both up- and down-regulate SFK activity in T cells (7, 27), it is possible that CD45 could also regulate PTPα phosphorylation indirectly by negatively regulating SFK activity. To determine whether SFK activity can regulate tyrosine 789 phosphorylation of PTPα, transfected CD45+ and CD45− T cells were treated with 10 μM PP2, a SFK inhibitor (28) and the phosphorylation state of PTPα examined. Fig. 2C indicates that the tyrosine phosphorylation of PTPα in CD45− T cells was significantly
reduced after 30 min of treatment with WP2 but not with the inactive PP2 analogue, PP3. These results implicate SFK activity in the phosphorylation of tyrosine 789.

Differential Effect of PTPα and PTPα-Y789F on the Phosphorylation State of Fyn and Cbp in CD45−T Cells—Having established that the presence of CD45 affects the phosphorylation state of tyrosine 789 in PTPα, we investigated whether the Y789F mutation, which prevents phosphorylation at this site, affected the function of PTPα. To do this, we examined the effect of PTPα and PTPα-Y789F in CD45+ and CD45−T cells.

As PTPα has been shown to regulate the SFK Fyn and phosphorylation of Cbp in thymocytes (16), we first examined these in the transfected CD45+ and CD45−T cells sorted for high expression of VSVG-tagged PTPα. Fyn was immunoprecipitated from parental PTPα and PTPα-Y789F transfected cells and its tyrosine phosphorylation state compared with that of Lck, the other SFK expressed in T cells. Both Fyn and Lck were hyperphosphorylated in the CD45−T cells compared with CD45+ T cells, consistent with a role for CD45 in dephosphorylating these SFKs, as has been described previously (29, 30). Overexpression of PTPα reduced the total phosphoryrosine level of Fyn and more specifically, the level of the negative regulatory phosphotyrosine 529 of Fyn in CD45−T cells (Fig. 3A), suggesting a positive role for PTPα in Fyn activation. However, analysis of Fyn activity in an in vitro kinase assay (Fig. 3B, top panel) revealed that the presence of PTPα reduced Fyn activity, suggesting that PTPα can also negatively regulate Fyn activity. Overexpression of PTPα in the CD45−T cells also reduced Fyn phosphorylation, indicating that both CD45 and PTPα can affect the phosphorylation state of Fyn. Interestingly, expression of PTPα-Y789F had a lesser effect on Fyn phosphorylation and Fyn activity compared with PTPα (Fig. 3A), even though expression levels of the two PTPs were similar (Fig. 3B, bottom panels). This demonstrates that PTPα and PTPα-Y789F differentially affect the phosphorylation state of Fyn in CD45+ and CD45−T cells and indicates that PTPα is more efficient at dephosphorylating Fyn than is PTPα-Y789F. In contrast, analysis of Lck immunoprecipitates from PTPα and PTPα-Y789F transfected cells did not reveal a major effect of either phosphatase on Lck phosphorylation (Fig. 3A), suggesting that Lck is not a major substrate for PTPα. Together, this suggests that PTPα acts primarily on Fyn in T cells.

Fyn has been implicated as a Cbp kinase (30), and we examined whether the altered in vitro activities of Fyn were consistent with the in vivo phosphorylation of this potential substrate. Indeed, immunoprecipitated Cbp from CD45−T cells exhibited enhanced phosphorylation and phosphorylation-dependent association with Csk, compared with Cbp from CD45+T cells, and the overexpression of PTPα reduced these increases (Fig. 3B, middle panels). Compared with PTPα, overexpressed mutant PTPα-Y789F was less effective in reducing Cbp phosphorylation and the extent of Cbp-Csk association in the CD45−T cells. These findings demonstrate that PTPα more potently promotes Fyn and Cbp dephosphorylation than does PTPα-Y789F.

PTPα-Y789F Is Less Efficient than PTPα in Restoring CD3-induced Phosphorylation in CD45−T Cells—Transfection of PTPα into CD45−T cells has previously been shown to par-

FIGURE 3. Effect of PTPα on the phosphorylation state of Fyn, Lck, and Cbp. A, Fyn and Lck were immunoprecipitated from equal amounts of protein from parental CD45+ and CD45−T cells as well as from PTPα-transfected (WT α) and PTPα-Y789F (Y789F) transfected CD45+ and CD45−T cells lysed in radioimmunoprecipitation assay buffer, and their phosphorylation states determined with the phosphotyrosine mAb 4G10 (PY). Alternatively, the membranes were stripped and reprobed with a phospho-site specific antisera (Biosource International) that recognizes the phosphorylated negative regulatory site of Fyn (pTyr529 Fyn). Membranes were then stripped and reprobed with Fyn or Lck antibodies to determine the amount of immunoprecipitated kinase. B, immunoprecipitated Fyn was assayed for in vitro kinase activity, and a representative autoradiograph is shown (top panel). Arrows indicate (from top to bottom) a phosphorylated Fyn-associated protein, Fyn, IgG, and enolase. Immunoprecipitated Cbp was probed with 4G10 (PY) to determine its level of tyrosine phosphorylation and was subsequently reprobed for the Cbp-associated kinase, Csk, and then for Cbp itself (three middle panels). The amounts of PTPα, VSVG-tagged PTPα, and CD45 in the cells were determined by immunoblotting cell lysates (three bottom panels). Experiments were repeated at least three times.
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The data thus far are consistent with the possibility that PTPα-Y789F is a less efficient phosphatase than PTPα. To further investigate this, CD44-mediated Pyk2 phosphorylation and T cell spreading were examined in the PTPα- and PTPα-Y789F-transfected T cells. Pyk2 is a member of the focal adhesion kinase family expressed in T cells and has been implicated in cell adhesion and migration processes. CD44-mediated Pyk2 phosphorylation is dependent on SFK activity, yet is negatively regulated by CD45 (9). This is opposite to the role of CD45 in TCR/CD3 signaling where it has a positive regulatory action on Lck and is required for efficient stimulation (reviewed in Refs. 3–5). Here, PTPα- and PTPα-Y789F-transfected CD45+ T cells were immobilized on CD44 antibody and cell spreading and Pyk2 phosphorylation compared with that of parental CD45+ T cells. PTPα-transfected cells showed reduced cell spreading compared with the inclusion of Pyk2 phosphorylation in TCR/CD3-mediated signaling in T cells. The data suggest that the reduced ability of PTPα to dephosphorylate PTPα-Y789F on Fyn, Cbp, and in restoring TCR/CD3 signaling events in CD45+ T cells suggests that PTPα-Y789F has a positive regulatory action on Lck and is required for efficient stimulation (reviewed in Refs. 3–5). Here, PTPα- and PTPα-Y789F-transfected CD45+ T cells were immobilized on CD44 antibody and cell spreading and Pyk2 phosphorylation compared with that of parental CD45+ T cells. PTPα-transfected cells showed reduced cell spreading compared with the

CD45+ T cells, and spreading was even more strongly reduced in PTPα-Y789F-expressing CD45+ T cells (Fig. 5A). Pyk2 phosphorylation was strongly induced in CD45+ T cells after 2 h but was reduced in PTPα-expressing cells and almost abolished in PTPα-Y789F-expressing cells (Fig. 5B), indicating a direct correlation between the extent of Pyk2 phosphorylation and cell spreading. Notably, PTPα-Y789F-transfected cells showed lower induced levels of Pyk2 phosphorylation than PTPα-transfected cells. This is opposite to the effect of PTPα and PTPα-Y789F on the phosphorylation state of Fyn and Cbp where lower phosphorylation of these proteins was observed in PTPα-transfected cells. This suggests that phosphorylation of PTPα at tyrosine 789 may differentially influence the dephosphorylation of specific substrates and raises the possibility that phosphorylation at tyrosine 789 may regulate the substrate specificity of PTPα.

To further explore the possible mechanism for reduced Pyk2 phosphorylation in PTPα-Y789F-transfected cells, immunoprecipitated Pyk2 was re-probed for associated PTPα. Surprisingly, PTPα but not PTPα-Y789F was found in the complex with Pyk2 both before and after CD44 stimulation (Fig. 5B). Furthermore, immunoprecipitation of PTPα and PTPα-Y789F at 0 and 2 h after CD44 stimulation revealed an association between Grb2 and PTPα, but not with PTPα-Y789F (Fig. 5C). Conversely, both PTPα and PTPα-Y789F associated with immunoprecipitated Fyn isolated from either CD45+ or CD45− T cells (Fig. 5D). This highlights two differences between PTPα and PTPα-Y789F, one in the ability of PTPα to co-immunoprecipitate Grb2 and the second in the ability to associate with Pyk2 in CD45+ T cells.

Collectively, these data identify tyrosine 789 in PTPα as a site of phosphorylation in T cells and demonstrate that phosphorylation at this site is negatively regulated by CD45. CD45 can directly dephosphorylate PTPα at tyrosine 789 in vitro, raising the possibility that PTPα might be a substrate for CD45 in T cells. PTPα-Y789F-transfected CD45− T cells showed a reduced ability to dephosphorylate Fyn and restore TCR/CD3 signaling events, compared with PTPα-transfected CD45− T cells, suggesting that phosphorylation of PTPα at tyrosine 789 may facilitate the dephosphorylation of Fyn. Conversely, PTPα-Y789F had the greatest effect on reducing Pyk2 phosphorylation and reducing T cell spreading in CD45− T cells. This highlights two differences between PTPα and PTPα-Y789F, one in the ability of PTPα to co-immunoprecipitate Grb2 and the second in the ability to associate with Pyk2 in CD45− T cells.

DISCUSSION

Although the role of CD45 in regulating Src family tyrosine kinases by regulating their phosphorylation is well established, its role in regulating protein tyrosine phosphatases by a similar mechanism has not previously been described. Here we provide evidence that PTPα is a target for CD45 in T cells. We establish that PTPα is phosphorylated at Tyr-789 in T cells and show that phosphorylation at this site is likely regulated by the balance of SFK and CD45 activities. In the absence of CD45, enhanced phosphorylation of PTPα is observed. This may be because of the dysregulation and enhanced activation of SFKs in the
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In non-hematopoietic cells (that lack CD45), about 20% of PTPα is constitutively phosphorylated at Tyr-789 (25), and PTPα Tyr-789 phosphorylation has been reported to play a role in PTPα-mediated mitotic activation of Src in fibroblasts (17, 33). Integrin stimulation in fibroblasts induces further phosphorylation of PTPα at Tyr-789, however this PTPα Tyr-789 phosphorylation is not required for integrin-induced PTPα-mediated SFK activation, although it is required for optimal cytoskeletal rearrangement and focal adhesion formation that determine cell migration (26). These contrasting results indicate that the phosphorylation of PTPα within its C-terminal tail can modulate PTPα function but that it may do so in a manner dependent upon signaling context. Here, the function of PTPα and the non-phosphorylated mutant PTPα-Y789F was compared in different signaling settings in T cells and revealed striking differences in their ability to affect the phosphorylation state of various potential PTPα substrates.

PTPα is expressed in various cell types and like CD45, is a well established regulator of SFKs (14). Recently, examination of thymocytes from PTPα-null mice revealed that Fyn and the potential Fyn substrate Cbp were hyperphosphorylated (16). Fyn was hyperphosphorylated at both the negative and positive regulatory sites in PTPα−/− thymocytes and was more active than Fyn from wild-type mouse thymocytes in an in vitro assay (16). Consistent with these findings, in the present study we observe that the overexpression of PTPα in CD45− T cells induces the dephosphorylation of Fyn and its substrate, Cbp. Fyn was dephosphorylated at the negative regulatory site yet its activity was decreased in an in vitro kinase activity. Interestingly, the mutant PTPα-Y789F was less effective than PTPα in causing Fyn and Cbp dephosphorylation and in reducing the in vitro kinase activity of Fyn, implicating a role for Tyr-789 phosphorylation in the regulation of Fyn by PTPα in T cells.

In contrast to its effect on Fyn, PTPα did not significantly affect the phosphorylation state of Lck, in accordance with the finding that Fyn but not Lck activity is altered in PTPα-null thymocytes (16). This may reflect a difference in location rather than a difference in substrate specificity as PTPα has been shown to specifically affect the activity of Fyn in lipid rafts (16). In that and in the present study, radioimmune precipitation assay buffer was used to solubilize Fyn from lipid rafts. This may explain why no major difference in Fyn phosphorylation was observed previously in PTPα-transfected CD45− T cells where Triton X-100 was used as the solubilizing agent (15). It is interesting to note that CD45 dramatically affects the phosphoryla-
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The location of these PTPs and SFKs is likely to play a role in their regulation and substrate specificity as CD45 and Lck are thought to reside primarily outside lipid rafts prior to T cell activation, whereas Fyn is enriched in lipid rafts along with the lipid raft resident protein, Cbp, and appears to be specifically regulated in rafts by a small population of raft-residing PTPα. However, these locations are not mutually exclusive as most of PTPα co-localizes with the majority of CD45 in the non-raft fraction (16), and only a minor population of CD45 can be detected in rafts (34), where it has been implicated in regulating Cbp phosphorylation (24). Cbp phosphorylation recruits Csk, a tyrosine kinase that phosphorylates the negative regulatory site of SFKs to counteract the activities of PTPα and CD45. Thus there appears to be an interacting network of self-regulating mechanisms designed to regulate SFK phosphorylation and prevent kinase overactivation.

Examination of TCR/CD3 signaling in PTPα-transfected CD45−/− cells also revealed a stronger ability of PTPα to restore TCR signaling compared with PTPα-Y789F. This correlates with the increased ability of wild-type PTPα to reduce Fyn and Cbp phosphorylation. Analysis of mice deficient in Lck, Fyn, or both SFKs has determined that Lck is the predominant SFK required to initiate TCR signaling in thymocytes and mature T cells. However, current models of TCR activation put Fyn downstream of Lck activation, with Lck translocating to lipid rafts upon TCR stimulation to activate raft-associated Fyn (35, 36) by an as yet undetermined mechanism. An intriguing possibility is that PTPα is a target of Lck, thus promoting PTPα function as a Lck effector and a Lck/Fyn signaling intermediate. Strikingly, PTPα and PTPα-Y789F exhibited reversed efficiencies in modulating events in another signaling pathway, that of CD44-induced cell spreading. Pyk2 has been identified as a Fyn substrate in T cells (37), and previous studies have shown that CD44-mediated Pyk2 phosphorylation is dependent upon SFK activity, is negatively regulated by CD45, and correlates well with T cell spreading (9). The expression of PTPα in CD45−/− cells demonstrated that like CD45, PTPα negatively affected CD44-mediated Pyk2 phosphorylation and cell spreading. However, in the CD44-stimulated T cells, PTPα-Y789F was more effective than PTPα in preventing Pyk2 phosphorylation and cell spreading. This suggests that either Pyk2 is a preferred substrate for PTPα-Y789F or that mutation at this site promotes access of the phosphatase to Pyk2. Alternatively, PTPα may be affecting Pyk2 phosphorylation indirectly, via its effect on Fyn. Either PTPα is more efficient at activating Fyn or PTPα-Y789F is more efficient at inactivating Fyn, by dephosphorylation at the autophosphorylation site. Given the fact that Fyn is able to phosphorylate Pyk2 in the absence of exogenous PTPα, we favor the explanation that PTPα-Y789F leads to the dephosphorylation of Pyk2, either directly or indirectly.

Interestingly, a clear difference in the abilities of PTPα and PTPα-Y789F to associate with Pyk2 and Grb2 was apparent both before and after CD44 stimulation. Mutation of Tyr-789 abolished the constitutive association observed between PTPα and Pyk2 and between PTPα and Grb2. The latter observation is in accordance with phospho-Tyr-789 being a Grb2-SH2 binding site (25, 38), but this is the first demonstration that PTPα can associate with Pyk2, although we do not know whether this interaction is direct or indirect. Grb2 has been shown to bind to Pyk2, although this was via Grb2 SH2 domain and phospho-Pyk2 (39). The complex of PTPα and Grb2 does not favor Pyk2 dephosphorylation implying that either Grb2 protects Pyk2 against dephosphorylation or that Grb2 hinders PTPα activity, as has been suggested (25, 38). Although further studies will be required to identify the precise mechanism, this clearly demonstrates that the mutant PTPα-Y789F can function differently to PTPα, which can be phosphorylated at this site. In addition, the modulatory effect of Tyr-789 is dependent on the signaling context in T cells.

Overall, these studies demonstrate the novel regulation of one PTP by another PTP. The data suggest that CD45 can both directly and indirectly affect the phosphorylation state of PTPα at Tyr-789 in T cells. We provide evidence that mutation at this site negatively affects the ability of PTPα to reduce the phosphorylation of Cbp and Fyn, to reduce the kinase activity of Fyn, and to restore TCR signaling events. Conversely, we find that mutation at this site enhances the ability of PTPα to reduce Pyk2 phosphorylation and CD44-mediated cell spreading. This study provides the first evidence in T cells that mutation of this phosphorylation site can differentially modulate the function of PTPα in T cells.

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