Expression and Catalytic Activity of the Tyrosine Phosphatase PTP1C Is Severely Impaired in Motheaten and Viable Motheaten Mice

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Summary

Mutations in the gene encoding the phosphotyrosine phosphatase PTP1C, a cytoplasmic protein containing a COOH-terminal catalytic and two NH_2-terminal Src homology 2 (SH2) domains, have been identified in motheaten (*me*) and viable motheaten (*me^v*) mice and are associated with severe hemopoietic dysregulation. The *me* mutation is predicted to result in termination of the PTP1C polypeptide within the first SH2 domain, whereas the *me^v* mutation creates an insertion or deletion in the phosphatase domain. No PTP1C RNA or protein could be detected in the hemopoietic tissues of *me* mice, nor could PTP1C phosphotyrosine phosphatase activity be isolated from cells homozygous for the *me* mutation. In contrast, mice homozygous for the less severe *me^v* mutation expressed levels of full-length PTP1C protein comparable to those detected in wild type mice and the SH2 domains of *me^v* PTP1C bound normally to phosphotyrosine-containing ligands in vitro. Nevertheless, the *me^v* mutation induced a marked reduction in PTP1C activity. These observations provide strong evidence that the motheaten phenotypic results from loss-of-function mutations in the PTP1C gene and imply a critical role for PTP1C in the regulation of hemopoietic differentiation and immune function.

Homozygosity for the motheaten (*me*)[1] or allelic viable motheaten (*me^v*) mutations is associated with early onset of a severe autoimmune and immunodeficiency disease that leads to death by age 3 or 9 wk, respectively (1, 2). The disease reflects the presence of multiple hemopoietic cell abnormalities which include a marked overexpansion of the autoantibody-secreting $CD5^+$ subpopulation of peripheral B cells (3), a paucity of both B cell progenitors in the bone marrow and conventional B cells in the periphery (4), impaired T cell and NK cell function (5, 6), and increased production and tissue accumulation of granulocytes and monocyte/macrophages (2, 7). All of these defects can be transferred by transplantation of *me* or *me^v* bone marrow into irradiated wild type mice (8, 9), suggesting that the *me* locus acts in a cell autonomous manner to regulate a critical aspect of hemopoietic cell proliferation and differentiation.

We have previously shown that *me* and *me^v* mice have mutations in a gene encoding PTP1C (10), an intracellular tyrosine phosphatase expressed predominantly in hemopoietic cells and also known as HCP, SH-PTP1, and SHP (11–14). PTP1C represents one of three cytoplasmic tyrosine phosphatases identified to date that contain two tandemly aligned Src homology 2 (SH2) domains, and which may therefore be involved in regulation of tyrosine kinase-mediated signaling cascades (15–18). In contrast to the normal PTP1C transcript, PTP1C mRNAs amplified by RT-PCR from *me* bone marrow cells have a 101-base pair frame-shift deletion in the coding region of the NH_2-terminal SH2 domain, while those amplified from *me^v* bone marrow have either a 15-base pair in-frame deletion or a 69-base pair in-frame insertion within the sequence encoding the PTP1C catalytic domain (10). These abnormalities were shown to arise by virtue of altered mRNA splicing in both the *me* and *me^v* PTP1C genes resulting from single base pair mutations which alter splice signal sequences. These genetic data have recently been confirmed by Shultz et al. (19) and together with the recent localization of the PTP1C gene to the vicinity of the *me* locus on mouse chromosome 6 (19, 20), suggest that mutation of the PTP1C gene underlies expression of the *me* and *me^v* phenotype. To explore this possibility, PTP1C function and expression have

[1] *Abbreviations used in this paper*: EGFR, epidermal growth factor receptor; GST, glutathione *S*-transferase; *me*, motheaten; *me^v*, viable motheaten; PDGF, platelet derived growth factor.

been examined in hemopoietic tissues of *me* and *me*^{v} mice. The resultant data reveal that PTP1C tyrosine phosphatase expression and catalytic activity are severely impaired in *me* and *me*^{v} mice and thus suggest strongly that the motheaten phenotype is caused by loss-of-function mutations in the PTP1C gene.

Materials and Methods

Mice. Mice for these studies were obtained by mating C57BL/6J *me*^{v}/+ and +/+ and C3HeBFeJ *me*/+ and +/+ breeding pairs originally provided by The Jackson Laboratory (Bar Harbor, ME).

PCR-based Assays of PTP1C Mutation. To detect the *me*^{v} PTP1C gene T→A transversion, a 120-bp pair genomic fragment encompassing the site of the mutation was amplified from tail DNA using the primers PTP1C1002–5′(5′-CAGGAGAACACTCGTGTCAT-3′) and PTP1C1122–3′(5′-TGTATGGTATTGAACAAGGACC-3′), 2.5 U Taq polymerase (Pharmacia, Baie d'Urfé, Que., Canada), and the following PCR conditions: 5 min 95°C, followed by 30 cycles of 1.5 min at 94°C, 1 min 58°C, 1 min 72°C, and 7 min 72°C (last cycle). 5 μl of PCR product were mixed with 5 pmol unlabeled primer PTP1C1028 (5′-GACTACCAGAGAGGTGGAGAAAGG-3′) and 1.0 U Taq polymerase and subjected to 10 cycles of PCR primer extension using the initial PCR conditions. 5 μl of extension product was then mixed with 5 pmol [γ^{32}-P]dATP end-labeled PTP1C1028 primer, 1.0 U Taq polymerase, and 100 μM dCTP, dGTP, dTTP, and dATP and subjected to a second round of PCR primer extensions. 2 μl of final reaction mixture was then electrophoresed over a 15% SDS-polyacrylamide gel at 80 W for 2 h, and the extension products visualized by autoradiography. To detect the *me* PTP1C gene C nucleotide deletion, a 118-base pair genomic fragment encompassing the site of the mutation was amplified from tail DNA using the primers PTP1C175-5′(5′-ACTTCTATGACCTGTACGGA-3′) and PTP1C293-3′(5′TACTTAAGGTGGATGATGGTGC-3′), 1.5 U Taq polymerase, and the PCR conditions described above. After TaqI digestion and electrophoresis at 150 V for 3 h on a 12% SDS-polyacrylamide gel, PCR products were visualized by ethidium bromide staining.

RNA Isolation and Analysis. Total cellular RNA was extracted from spleen, thymus, and bone marrow tissue obtained from normal and mutant mice as previously described (21). For Northern analysis, 20 μg RNA samples were electrophoresed over 1% agarose-formaldehyde gels, transferred to GeneScreen Plus membranes (New England Nuclear, Mississauga, Ont., Canada), and hybridized with a 1-kb PCR fragment amplified from the PTP1C transcript phosphatase region (10). After autoradiography, blots were stripped and rehybridized with a 300-base pair β-actin gene fragment.

GST-PTP1C Fusion Proteins. To generate glutathione *S*-transferase (GST)-PTP1C SH2 domain fusion proteins, DNA fragments encompassing the two PTP1C SH2 domains (amino acids 1–296) were PCR amplified from wild type–, *me*^{v}- and *me*-derived PTP1C cDNA template using oligonucleotide primers containing the appropriate restriction sites and complementary to the sequences flanking the SH2 domain region (10). After EcoRI/BamHI digestion, the amplified products were cloned into pGEX-2T (Pharmacia), transformed into DH5α *Escherichia coli* and the GST fusion proteins were purified from bacterial lysates as previously described (22).

Antibodies. Polyclonal anti-PTP1C and anti-Syp antibodies were generated by injecting rabbits with 100 μg wild type GST-PTP1C SH2 domain fusion protein or with a GST fusion protein containing the two SH2 domains (residues 2–216) of Syp as previously described (16).

Immunoblotting Analysis. Total cellular protein lysates were prepared by resuspending freshly isolated bone marrow, spleen, or thymic tissues in 1 ml lysis buffer (phosphate buffered saline, pH 7.0, containing 1% Triton X-100, 1% Tween, 1 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 0.001 mM DTT). 100 μg of total cell lysate protein was electrophoresed through 10% SDS-polyacrylamide, electroblotted onto nitrocellulose membranes, and the proteins were detected by immunoblotting with polyclonal rabbit anti-PTP1C SH2 or anti-Syp antibodies (1:1,000 dilution) followed by ^{125}I-protein A as previously described (23).

PTP1C Immunoprecipitation and Phosphatase Activity. 100 μg total spleen or liver cell lysate protein was incubated for 2 h at 4°C with 20 μl anti-PTP1C SH2 or anti-Syp SH2 antibodies, respectively, and 100 μl of protein A-Sepharose (Pharmacia). Immune complexes were collected by centrifugation (5 min 10,000 *g*) and one half of each sample was washed three times with lysis buffer, pelleted, boiled for 5 min in SDS-sample buffer, and electrophoresed over 10% polyacrylamide. After transfer to nitrocellulose, specific proteins were detected by immunoblotting with the anti-PTP1C or anti-Syp antibodies followed by ^{125}I-protein A. For phosphatase assays, portions of the anti-PTP1C and anti-Syp immunoprecipitates were incubated in reaction buffer (24) at 37°C with 2 mM *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO). Reactions were terminated by addition of 1 ml 200 mM NaOH and absorbance at 410 mM was determined.

In Vitro Binding Assays. For binding assays, Rat-1 cells (R1hER) overexpressing the human epidermal growth factor receptor (EGFR) and Rat-2 fibroblasts were grown in Dulbecco's modified Eagle medium supplemented with 10% FBS and then serum-starved for 48 h. Quiescent R1hER and Rat-2 cells were either left unstimulated or stimulated for 5 min at 37°C with EGF (80 ng/ml) or platelet derived growth factor (PDGF, 75 ng/ml), respectively (Upstate Biotechnology, Inc., Lake Placid, NY). After lysis, aliquots of the cell lysates were incubated for 1 h at 4°C with 5 μg purified wild type, *me*^{v} or *me* GST-PTP1C SH2 domain fusion proteins immobilized on glutathione-agarose beads. After several washes in lysis buffer, complexes as well as remaining aliquots of untreated cell lysates were electrophoresed through 10% SDS-polyacrylamide and analyzed by immunoblotting with either anti-EGFR or anti-PDGFR antibodies (Upstate Biotechnology Inc.) and ^{125}I-protein A.

Results and Discussion

With the discovery of point mutations in the PTP1C gene of both *me* and *me*^{v} mice (10), the possibility emerged that defective function of this hemopoietic tyrosine phosphatase might underlie expression of the motheaten phenotype. To confirm the correlation between this phenotype and PTP1C mutation, the segregation of the normal and mutant PTP1C alleles was examined in offspring derived from mating heterozygous *me* and *me*^{v} mice. For these analyses, a PCR-based strategy predicated on differential termination of primer extension (25) was used to distinguish the T→A transversion-containing *me*^{v} mutant PTP1C allele from the normal allele; these appear as 31- and 30-base pair extension products, respectively (Fig. 1 *A*). The *me* mutant PTP1C allele was identified in PCR amplified genomic DNA by taking advantage of the loss of a TaqI restriction site consequent to the C nucleotide deletion (at position 228) that demarcates this mutant allele

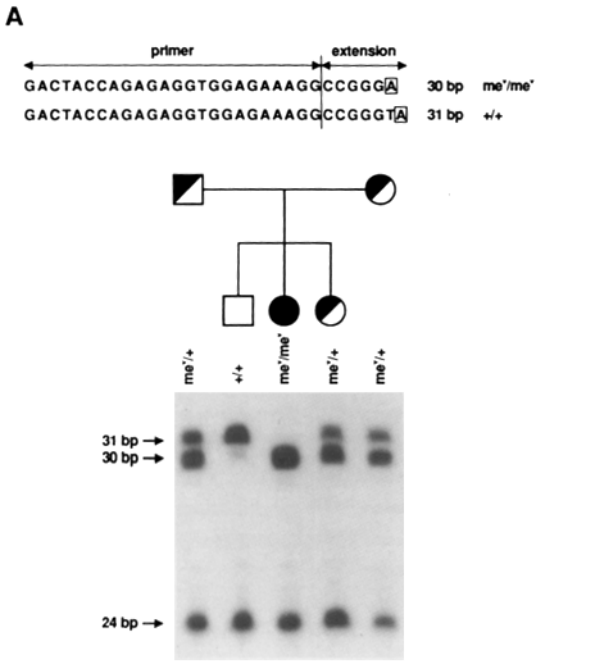

Figure 1. Detection of the PTP1C gene mutations in *me*^{v} and *me* mice. (*A*) A 120-base pair genomic fragment encompassing the site of the T→A transversion in the *me*^{v} PTP1C gene was PCR amplified from C57BL/6J *me*^{v}/+, *me*^{v}/*me*^{v}, and +/+ tail DNA and subjected to two rounds of PCR primer extension as described in Materials and Methods. Termination of extension at the first adenine 3' to the primer (*boxed residues*) results in two radiolabeled extension products, a 30-nucleotide (nt) fragment (upper sequence) and a 31 nt fragment (lower sequence) that represent the *me*^{v} and normal PTP1C alleles, respectively. The radiograph shows the extension products obtained from +/+ (31 nt only), *me*^{v}/*me*^{v} (30 nt only) and *me*^{v}/+ (both 31 and 30 nt) mice. (*B*) A 118-base pair genomic fragment encompassing the site of the C deletion (nt position 228) in the *me* PTP1C gene was PCR amplified from tail DNA of parents and offspring of one C3HeBFeJ *me*/+ × *me*/+ cross as described in Materials and Methods. PCR products were digested with TaqI, electrophoresed over a 12% SDS-polyacrylamide gel, and visualized by ethidium bromide staining. The autoradiograph shows the association of the normal PTP1C allele with two TaqI restriction fragments of 53 bp and 65 bp, the *me* PTP1C allele with an intact 118-bp fragment and the *me* heterozygous state with all three fragments.

(Fig. 1 *B*). The results of segregation analyses using these two assays (Fig. 1) confirm the presence of point mutations in the *me* and *me*^{v} PTP1C genes and indicate that the methods permit detection of the PTP1C mutant and normal alleles and resolution of the *me* and *me*^{v} heterozygous states.

PTP1C expression was initially examined in normal and mutant mice by Northern blot analysis and the results confirmed previous data (12–14) showing expression to be prominent in hemopoietic tissues (Fig. 2) and negligible in most other tissues of normal mice (not shown). The results also confirm recent data by Shultz et al. (19) showing that PTP1C RNA expression is comparable in *me*^{v} and normal bone marrow, but not detectable in *me* marrow cells (Fig. 2). Moreover, the data shown in Fig. 2 reveal levels of PTP1C transcript to be normal in *me*^{v} spleen and thymus, while PTP1C RNA is undetectable in any hemopoietic tissue isolated from *me* mice. The failure to detect this transcript in *me* tissues, in contrast to detection of apparently normal PTP1C transcript levels in *me*^{v} tissues, is consistent with the more severe nature of both the PTP1C mutation and clinical phenotype found in *me* mice.

Analysis of PTP1C protein expression using a polyclonal anti-PTP1C SH2 domain antibody revealed the presence of two distinct PTP1C species (~67 and 70 kD, respectively) in various hemopoietic tissues of both C57BL/6J and C3HeBFeJ wild type mice (Fig. 3, *A* and *B*). As anticipated from the analysis of *me* and *me*^{v} PTP1C RNA sequences, neither of these PTP1C molecules were detected in tissues of *me* mice (Fig. 3 *B*), while PTP1C species comparable in size to wild type PTP1C were apparent in the *me*^{v} samples (Fig. 3 *A*). However, in contrast to normal mice, the *me*^{v} mice also displayed a lower molecular weight PTP1C species of about 34 kD. As the 34-kD band was not detected using an anti-peptide antibody raised against a 14 residue segment of the PTP1C COOH-terminal tail (not shown), it likely

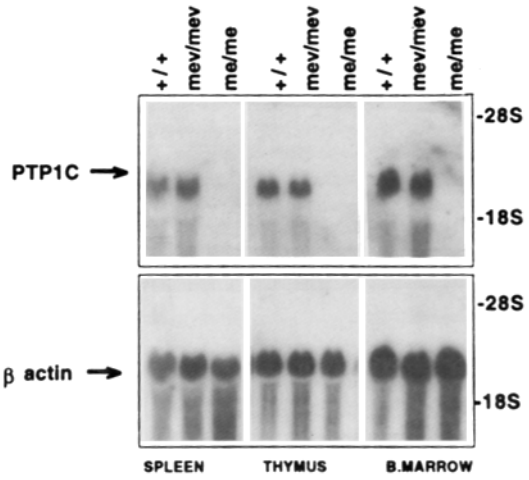

Figure 2. Northern blot analysis of PTP1C expression in *me* and *me*^{v} hemopoietic tissues. Total cellular RNA was extracted from spleen, thymus, and bone marrow of C57BL/6J wild type, *me*^{v} homozygous and *me* homozygous mice and 20 μg samples were electrophoresed over 1% agarose-formaldehyde gels and transferred to GeneScreen Plus membranes. Membranes were hybridized with a 1-kb PCR amplified fragment from the PTP1C cDNA (*top*) and the blots then stripped and reprobed with a 300-base pair β-actin gene fragment (*bottom*). Positions of the 28S and 18S rRNAs are shown on the right and the PTP1C and β-actin transcripts on the left.

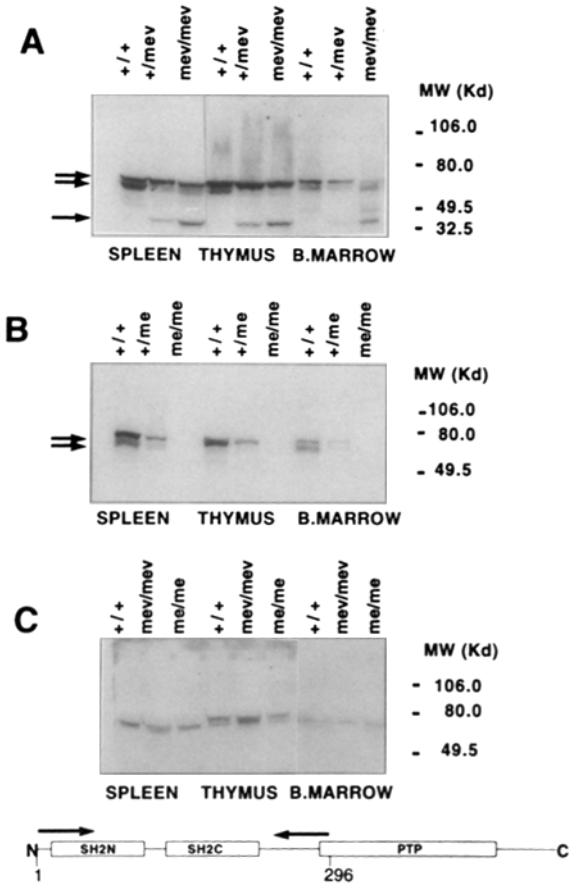

Figure 3. Immunoblot analysis showing PTP1C expression in various *me* and *me*^{v} tissues. Cell lysates were prepared from the indicated tissues of (*A*) C57BL/6J wild type and congenic *me*^{v} heterozygous and homozygous mice and (*B*) C3HeBFeJ wild type, and congenic *me* heterozygous and homozygous mice. 100 μg of total cell lysate protein was electrophoresed through SDS-PAGE, transferred to nitrocellulose, and immunoblotted with rabbit polyclonal anti-PTP1C (*A* and *B*) or anti-Syp (*C*) antibodies and ^{125}I-protein A. The positions of molecular mass markers are shown on the right and of various PTP1C species on the left (*arrows*). The longer arrows over the schematic diagram of PTP1C predicted protein structure (*bottom*) indicate the relative positions of PCR primers used to derive the PTP1C SH2-domain encoding fragment (amino acids 1–296) that was cloned into the pGEX expression vector to obtain a GST-PTP1C fusion protein for derivation of anti-PTP1C SH2 antibodies.

represents a fragment from the NH_2-terminal portion of the protein. Whether this fragment arises by degradation of one or both of the *me*^{v} mutant proteins remains to be determined, but its presence in *me*^{v} homozygous and, at a reduced level, in *me*^{v} heterozygous mice, confirms the genetic data suggesting that *me*^{v} mutants do not produce normal PTP1C protein. Similarly, the failure to detect PTP1C in *me* tissues using either the anti-carboxy tail peptide (not shown) or the anti-SH2 domain antibodies, and the detection of about 50% less PTP1C protein in *me* heterozygous relative to wild type mice, are consistent with the previous finding of a frame shift mutation in the *me* PTP1C coding region that would predictably abrogate expression of the protein. By contrast, the expression of Syp, a ubiquitously expressed cytoplasmic tyrosine phosphatase structurally similar to PTP1C (16), was normal in both *me* and *me*^{v} mice (Fig. 3 *C*). Taken together, these results argue for a causal relationship between PTP1C mutation and expression of the motheaten phenotype.

While previous studies of PTP1C expression in normal mice have generally identified only a single 64–68-kD PTP1C species (14, 19, 26), the wild type mice studied here clearly express two distinct PTP1C species (Fig. 3, *A* and *B*). This latter result is consistent with our previous data showing that alternative splicing of a 39-amino acid segment within the PTP1C COOH-terminal SH2 domain results in expression of two distinct PTP1C transcripts in normal bone marrow (10). The two isoforms may therefore represent the products of different PTP1C splice variants. The detection of only a single PTP1C species in the C3HeBFeJ wild type thymic cells studied here (Fig. 3 *B*) and in the C57BL/6J bone marrow macrophages studied by Shultz et al. (19), as well as recent data on PTP1C expression in human tumor cell lines (27), raise the possibility that PTP1C isoforms may be differentially expressed in various cell types and possibly among different inbred strains.

PTP1C catalytic activity was also assessed in the mutant mice and, as anticipated, no anti-PTP1C immunoprecipitable phosphatase activity was detected in the *me* spleen cells (Fig. 4 *A*). Although in a previous study, some PTP1C catalytic activity was detected in *me* macrophages (19), the lack of any such activity in the *me* cells studied here is consistent with the absence of immunoprecipitable PTP1C protein in these mice. Similarly, while the amount of protein immunoprecipitated from *me*^{v} spleen cells with anti-PTP1C antibody appeared comparable to that obtained from normal cells, the phosphatase activity associated with PTP1C immunoprecipitated from *me*^{v} cells was markedly reduced relative to PTP1C from normal cells. By comparison, the phosphatase activities of the Syp tyrosine phosphatase immunoprecipitated from *me*, *me*^{v}, and wild type liver, were essentially the same (Fig. 4 *B*). PTP1C protein is therefore not only abnormally expressed in *me* and *me*^{v} hemopoietic cells, but is also dysfunctional in these cells. It is important to note, although full-length PTP1C polypeptides are expressed in *me*^{v} cells, these proteins are enzymatically impaired, presumably as a result of the structural alterations introduced into the phosphatase domain by the *me*^{v} mutation.

To examine the effect of the *me* and *me*^{v} mutations on PTP1C SH2 domain function, PTP1C mRNA segments containing the two adjacent SH2 domains were amplified by reverse transcriptase (RT)-PCR from *me* and *me*^{v} bone marrow cells and expressed in bacteria as GST fusion proteins. Although the spectrum of proteins interacting with PTP1C SH2 domains have not been identified, association of the PTP1C SH2 domain–containing region with activated EGFR has been demonstrated in vitro (11, 17). Accordingly, GST-PTP1C fusion proteins coupled to glutathione-Sepharose were incubated with cell extracts from EGF-stimulated R1hER fibroblasts or PDGF-stimulated Rat-2 cells and the bound receptors were detected by immunoblotting with anti-EGFR or anti-PDGFR antibodies, respectively. As shown in Fig. 5, the *me*^{v} PTP1C SH2 domain fusion protein, which is identical in sequence to that derived from the wild type PTP1C SH2 domain segment, associated strongly with the au-

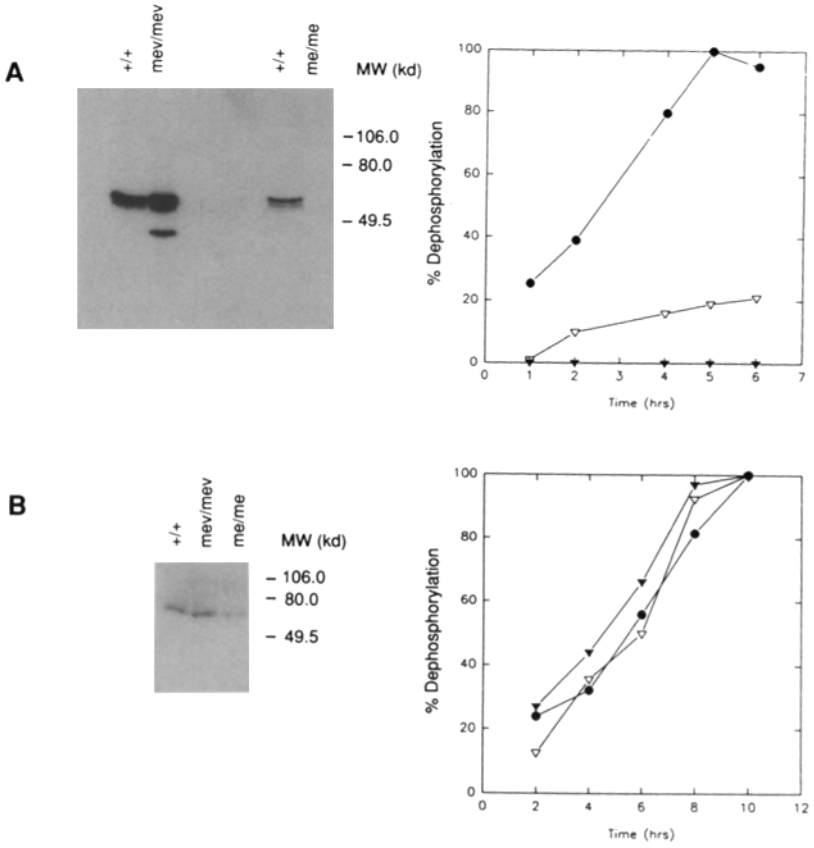

Figure 4. Tyrosine phosphatase activities of immunoprecipitated PTP1C and Syp from normal, *me* and *me*^{v} mice. Cell lysates were prepared from spleen (*A*) and liver (*B*) of *me*^{v}, *me*, and congenic wild type mice and 100 μg total cell lysate protein was immunoprecipitated with protein A-Sepharose beads and anti-PTP1C SH2 (*A*) or anti-Syp SH2 (*B*) antibodies as described in Materials and Methods. Aliquots of the precipitates were electrophoresed through a 10% SDS-polyacrylamide gel, transferred to nitrocellulose, and blotted with anti-PTP1C (*A*) or anti-Syp (*B*) antibodies followed by ^{125}I-protein (*A*) Positions of molecular mass standards are shown on the right. Remaining aliquots of the anti-PTP1C (*A*) and anti-Syp (*B*) immunoprecipitates obtained from wild type (●), *me*^{v} (△), and *me* (▼) tissues were incubated with 2 mM *p*-nitrophenyl phosphate at 37°C for varying times and after addition of NaOH, absorbance was measured at 410 nM as described in Materials and Methods. The results (*upper and lower graphs on the right*) are expressed as percentages relative to the maximum OD 410 nm values obtained in wild type (●) tissues (i.e., % dephosphorylation).

tophosphorylated EGFR and PDGFR in this in vitro binding assay. Similarly, interaction between the *me*^{v}-derived PTP1C fusion protein and the PDGR expressed in PDGF-stimulated RAT-2 cells was apparent. By contrast, the *me* SH2 domain–containing fusion protein, which was less than one half

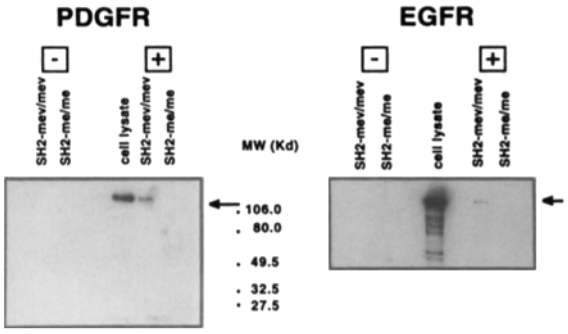

Figure 5. Association of PTP1C SH2 domains with activated EGF and PDGF receptors. Serum-starved Rat-2 (*A*) and R1hER fibroblasts (*B*) were left unstimulated (−) or stimulated (+) for 5 min with PDGF (*A*) or EGF (*B*). After cold lysis, aliquots of cell lysates were incubated with viable motheaten (SH2 *me*^{v}/*me*^{v})- and motheaten (SH2 *me*/*me*)–derived GST-PTP1C SH2 domain fusion proteins bound to glutathione-agarose beads. Complexes as well as remaining aliquots of lysates (*cell lysates*) were electrophoresed through SDS-PAGE and subjected to immunoblotting with anti-PDGFR (*A*) and anti-EGFR (*B*) antibodies and ^{125}I-protein A. The positions of molecular mass standards and PDGF and EGF receptors are indicated.

the size of the wild type and *me*^{v} SH2 domain fusion proteins (not shown), did not bind to either of these activated receptors (Fig. 5). These results provide further evidence that one or both of the PTP1C SH2 domains can interact with autophosphorylated forms of these receptors, and that PTP1C function is entirely abrogated by the *me* mutation.

As suggested by the finding of mutations in the *me* and *me*^{v} PTP1C gene and transcripts, the demonstration here that catalytically active PTP1C is not detectable in *me* mice and is greatly diminished in *me*^{v} mice argues that the motheaten phenotype results from loss-of-function mutations in the PTP1C gene. The more significant impairment in PTP1C tyrosine phosphatase activity seen in *me* compared to *me*^{v} mice provides a biochemical explanation for the differences in disease severity conferred by these allelic mutations. These findings, in conjunction with the plethora of hematologic cell defects manifested by *me* and *me*^{v} mice, indicate that PTP1C is critical to the differentiation of multiple hemopoietic lineages and imply that other cytoplasmic tyrosine phosphatases, such as Syp, do not compensate for the absence of PTP1C catalytic activity in these cells.

The presence in PTP1C of two SH2 domain elements suggests that this phosphatase can bind to particular tyrosine phosphorylated receptors and/or cytoplasmic proteins and that its influence on hemopoietic cell differentiation and function may reflect regulatory effects on a subset of signaling proteins. This possibility is supported by data implicating the structurally similar intracellular tyrosine phosphatase, *cork-*

screw, in the signal transduction pathway elicited by activation of the Drosophila torso receptor tyrosine kinase (18). While the specific targets for PTP1C binding and tyrosine dephosphorylation are unknown, PTP1C can associate with activated EGFR and PDGFR and dephosphorylate these and the hER-2-neu (17) receptors in vitro. These observations may be of physiologic relevance, as epithelial and particularly malignant epithelial cells, from which PTP1C was initially cloned (11), represent the major nonhemopoietic lineage that expresses substantial levels of PTP1C. Of more potential significance in relation to the *me* phenotype, are recent data showing that PTP1C dephosphorylates the Kit and CSF-1 receptors in vitro (28) and that tyrosine phosphorylation and increased expression of PTP1C are induced in conjunction with phorbol ester–stimulated macrophage differentiation (27) and CSF-1–stimulated macrophage activation, respectively. These tyrosine kinase receptors have been implicated in the induction of early hemopoietic differentiation (29) and development and function of mononuclear phagocytes (30), respectively, and thus a role for PTP1C in regulating signal transduction pathways activated by these receptors is consistent with the hemopoietic cell dysfunction associated with the *me* and me^v PTP1C mutations. Although the participation of PTP1C in these specific pathways in vivo is currently hypothetical, the increases in proliferation, spontaneous CSF-1 production and consequent tissue accumulation of the macrophage/monocyte population associated with loss of PTP1C function in motheaten mice, are consistent with a role for PTP1C in modulating CSF-1 receptor-mediated cell responses and raise the possibility that PTP1C functions as a negative regulator of the transduction pathways coupling the CSF-1 and possibly other tyrosine kinase receptors to cell proliferation. Similarly, other phenotypic consequences of PTP1C mutation such as expansion of erythroid, myeloid, and $CD5^+$ B lymphoid lineage cells, suggest that PTP1C may directly or indirectly negatively regulate proliferation and growth of a number of hemopoietic cell lineages. The availability of mice having little or no PTP1C catalytic activity provides an excellent opportunity to address this possibility and to identify the signaling pathways and molecular interactions that link PTP1C to expression of normal hemopoietic differentiation and immune function.

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