Identification of Novel Protein Tyrosine Phosphatases of Hematopoietic Cells by Polymerase Chain Reaction Amplification

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Polymerase chain reaction (PCR) conditions were used to amplify cDNAs that encode putative protein tyrosine phosphatases (PTPs) from a murine interleukin-3-dependent myeloid cell line. Primers for the reactions were based on conserved sequences of the catalytic domain that are shared among all known PTPs. Sequencing of 100 PCR-amplified cDNA clones identified seven different cDNA sequences. Two of these sequences were identical to the murine PTP genes Ly5/CD45/LCA and LRP/R-PTP-α. Two of the cDNA sequences were 95% identical to human PTP e (HPTPe) and rat brain PTP (PTP1B), respectively, and are likely to represent their murine homologs. Three of the cDNA sequences encoded novel potential PTPs. One of the putative PTPs was ubiquitously expressed while a second was predominately expressed in brain, kidney, and liver and at much lower levels in a variety of other cell types and tissues. The third novel putative phosphatase was expressed primarily in hematopoietic cells and tissues in a pattern that was comparable with Ly5/CD45/LCA. Further characterization of these novel PTPs will provide insights into the growth regulation of hematopoietic cells.

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A ROLE FOR PROTEIN tyrosine phosphorylation in growth regulation was first suggested by the demonstration of protein tyrosine kinase (PTK) activity of transforming genes. Subsequently, it was found that a number of growth factor receptors contained intrinsic PTK activity that was activated after ligand binding. However, a role for tyrosine phosphorylation in the regulation of hematopoietic cell growth has been more speculative because most of the cloned hematopoietic growth factor receptors do not have intrinsic PTK activity, including the receptors for interleukin-2 (IL-2), IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and erythropoietin (Epo). Nevertheless, several observations suggest that protein tyrosine phosphorylation is involved in hematopoietic cell growth. First, the binding of hematopoietic growth factors, including IL-3, GM-CSF, IL-2, and Epo, to their cognate receptors rapidly induces tyrosine phosphorylation of common substrates. Second, introduction of kinases that are responsible for the tyrosine phosphorylation of activated PTKs can abrogate the requirements of hematopoietic cells for their growth factors. Third, treatment of IL-3-dependent hematopoietic cells with the PTK-specific inhibitor, genistein, inhibits proliferation (O. Miura and J.N. Ihle, unpublished data). In general, the kinases that are responsible for the tyrosine phosphorylation that is seen after IL-3, GM-CSF, IL-2, or Epo binding are not known. However, in T cells, IL-2 binding has been shown to increase the PTK activity of the c-src-related tyrosine kinase c-lk.19

The ability to sustain a biologic response after growth factor binding and induction of tyrosine phosphorylation can also be speculated to be dependent on the rate that dephosphorylation occurs. Thus, protein tyrosine phosphatases (PTPs) may play a central role in limiting the growth response. Consistent with this, overexpression of PTPs can suppress the biologic response to insulin20 or the proliferative response to platelet-derived growth factor.21 Conversely, phosphatase inhibitors have been shown to potentiate the transforming activity of c-fes22 and can partially replace the requirements of growth factor-dependent cells for IL-3.23

Alternatively, PTPs may induce a growth response. In particular, dephosphorylation has been implicated in activation of T cells through the T-cell receptor (TCR) by the observation that T cells that lack the PTP CD45 fail to respond through the TCR in either proliferation or tyrosine phosphorylation. Moreover, the mutant cells require higher concentrations of IL-2 to obtain growth that is comparable with parental cells. In T cells, it has been hypothesized that CD45 may activate the kinase activity of c-lk, which associates with CD4 or CD8, with, or c-fyn, which associates with the TCR,23 by dephosphorylation of the carboxyl inhibitory site of tyrosine phosphorylation. Similarly, PTPs can activate the kinase activity of cdk2 kinase by dephosphorylation of Y15 and can induce cell cycle progression in yeast or Xenopus oocytes.

Experiments to evaluate the role of protein tyrosine dephosphorylation have been made possible by the recent purification and subsequent cloning of PTPs. Initially, a low molecular weight PTP (PTP1B) was isolated from human placenta, its amino acid sequence determined, and the gene subsequently cloned.35,36 The sequence had significant homology to the cytoplasmic domain of CD45 that was subsequently shown to have PTP activity.38,39 Based on sequence homology, a number of additional PTPs have been cloned. The known mammalian PTPs have been grouped into three classes. One class is cytoplasmic and includes PTP1B and TCPTP. The second and third classes are receptor-like phosphatases that contain a single phosphatase domain (human PTPB [HPTPB]) or tandem domains (CD45/LCA/Ly5, LAR, HPTPa/R-PTP-α/
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Materials and Methods

Tissues, cells, and cell culture. Balb/c mice of 12 weeks of age were killed and the organs collected. The tissues were immediately frozen in liquid nitrogen and used for the isolation of RNA. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) and murine IL-3 (25 U/ml) for IL-3-dependent cells. Mouse bone marrow-derived monocytes (BMM) were grown as previously described. The properties of the cell lines used in these studies have been previously described. 49

RNA isolation and Northern hybridization. Total cellular RNA and poly(A)+ RNA were isolated from mouse tissues, organs, and cell lines following techniques that have been previously described. 49 Approximately 20 μg of total cellular RNA or 5 μg of poly(A)+ RNA of each sample were separated in 1.2% agarose formaldehyde gels by electrophoresis and were blotted to nitrocellulose filters. The filters were hybridized with 32P-labeled random primed cDNA fragments and were detected by autoradiography.

PCR amplification. Two sets of oligonucleotide primers (PTP1 and PTP2) were synthesized and purified (Operon, Alameda, CA). The sequence of oligonucleotides is shown in Fig 1B. For cloning, cDNA was derived from poly(A)+ RNA of DA-3 cells by using a cDNA kit (Amersham, Arlington Heights, IL) and following the manufacturer’s instructions. Approximately 10 ng of cDNA was then amplified for 30 cycles with the PTP1/PTP2 primers using a Geneamp kit (Perkin Elmer Cetus, Norwalk, CT) using cycles of 92°C for 1 minute, 37°C for 1 minute, and 65°C for 2 minutes. The amplified cDNA was digested with SalI and BamHI enzymes, cloned into Bluescript vector (Stratagene, La Jolla, CA) and the sequence was determined by chain termination as previously described.

RESULTS

PCR amplification of PTP cDNAs. To identify novel PTP genes in IL-3-dependent hematopoietic cells, we used PCR amplification. This procedure takes advantage of the observation that all the known PTPs contain conserved regions in the carboxy-terminus that encode the catalytic domain. The consensus sequences for this region are shown in Fig 1, in which the carboxyl amino acid sequences of known PTPs are aligned. The alignments identified two consensus sequences, PTP1 (D/HFW-M-WE/Q) and PTP2 (HCSAGV/IG), that were selected to direct the synthesis of oligonucleotide primers. To reduce the number of oligonucleotides required for the full representation of the sequences, inosine was used at positions of amino acid variations. SalI and BamHI restriction sites were introduced at the ends of PTP1 and PTP2, respectively, to facilitate subsequent cloning.

Sequence comparison of PCR identified PTP cDNAs. The oligonucleotide primers were used to amplify cDNA clones from the DA-3 IL-3-dependent, murine myeloid leukemia cell line. These cells have a myeloblastic morphology and express cell surface markers normally expressed during early myeloid differentiation. One hundred PCR-amplified cDNA clones were isolated and sequenced and were found to represent one of seven cDNA sequences. As illustrated in Fig 2, all seven cDNA sequences contained the consensus sequences found in all PTPs. The number of isolates of each of the sequences is indicated in parentheses in the figure. By comparison of the non-primer-derived sequences

Fig 1. Identification of conserved amino acid sequences in the catalytic domains of PTPs. (A) The amino acid sequences for the first phosphatase domains of the indicated PTP are shown and were obtained from the following references: CD45,5,11 LAR,12 LRP, HPTPe,46 HPTPy/R-PTP-γ,40,46 or HPTP/R-PTP-δ.46

Fig 2. Sequence comparison of PCR identified PTP cDNAs. The oligonucleotide primers were used to amplify cDNA clones from the DA-3 IL-3-dependent, murine myeloid leukemia cell line. These cells have a myeloblastic morphology and express cell surface markers normally expressed during early myeloid differentiation. One hundred PCR-amplified cDNA clones were isolated and sequenced and were found to represent one of seven cDNA sequences. As illustrated in Fig 2, all seven cDNA sequences contained the consensus sequences found in all PTPs. The number of isolates of each of the sequences is indicated in parentheses in the figure. By comparison of the non-primer-derived sequences

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with the Genbank nucleotide sequence data bank, there were highly homologous or identical to known PTPs while three were novel cDNA sequences. In particular, PTPTY1 is identical to the first phosphatase domain of the murine PTP Ly5, which is the murine homologue of the human CD45/LCA gene.42 PTPTY28 is identical to the first phosphatase domain of the murine PTP termed LRPα or R-PTP-α,44 which is the homolog of the human HPTPα/R-PTP-α.44 PTPTY8 is 95% homologous in amino acid sequence to the first phosphatase domain of the human PTP HPTPe46 and therefore is likely to be the murine counterpart. Similarly, PTPTY20 was highly homologous in amino acid sequence (95%) to the first phosphatase domain of the cytosolic PTP1B from rat brain and is likely to be the murine homolog.

Among the cDNA clones that were isolated, PTPTY35, PTPTY42, and PTPTY43 were only distantly related to the known PTPs and are likely to represent novel genes. Recent comparisons of PTPs have been able to establish a phylogenetic tree that divides the 12 known PTPs into seven subgroups.48 Based on the sequence similarities with these groups, PTPTY35 is most homologous (52%) to the HPTPβ subgroup. PTPTY42 and PTPTY43 show low homologies of 35% to 40% to all the subgroups and with each other, suggesting that each may belong to a new and distinct subgroup.

Differential expression of hematopoietic PTP in fibroblast, lymphoid, and myeloid cell lines. To determine the cell lineage specificity for expression of the PTPs, the PCR-derived cDNAs were used to probe Northern blots of RNA from several cell lines that included T cells (DA-2), immature B cells (DA-8), mature B cells (NFS-112), immature myeloid cells (DA-3), macrophages (Bac1.2F5), and fibroblasts (NIH 3T3). As illustrated in Fig 3, the Ly5/CD45 (PTPTY1) probe detected transcripts of 6, 5.5, and 4.8 kb in myeloid and lymphoid cells but not in fibroblasts. This pattern is consistent with previous reports on the lineage specificity for expression of the Ly5/CD45/LCA gene.42 Similarly, probes for the LRP/R-PTP-α gene (PTPTY28) detected two transcripts of 3.5 and 3.8 kb in all the cells examined, consistent with previous studies.4445

Probes for the potential murine homolog of the rat PTP1B gene (PTPTY20), detected two transcripts of 4.3 and 2.0 kb in all the cell lines examined. This finding is consistent with previous studies that showed the expression of PTP1B transcripts of 4.3 and 2.0 kb in brain, liver, kidney, and spleen.47 Probes for the potential murine homolog (PTPTY8) of the human placental PTK HPTPe detected transcripts of 8 kb and 5.2 kb in fibroblasts, myeloid cells, macrophages, and T cells but not in the two B-cell lines. The pattern of expression of HPTPe has not been previously examined.

Among the novel putative PTP genes only the PTPTY42 gene showed a restricted pattern of expression. As shown in Fig 3, probes for PTPTY42 detected a transcript of approximately 2.6 kb in all the myeloid and lymphoid cell lines but did not detect transcripts in fibroblasts. Curiously, the PTPTY42 transcripts of DA-3 and DA-2 cells appeared slightly larger than the transcripts detected in DA-8, NFS-112, and Bac1.2F5 cells.

The two other novel PTPs were expressed at comparable levels in all the cells examined. As shown in Fig 3, probes for PTPTY35 detected a major transcript of 15 kb and a minor transcript of 8 kb. Lastly, PTPTY43-specific probe identified a single transcript of approximately 3.6 kb.

Differential expression of PTP genes in murine tissues. To further examine the pattern of expression of the PTP genes, Northern blots of various murine tissues were probed. As illustrated in Fig 4, the patterns of expression of PTPTY1 (CD45), PTPTY28 (LRP/R-PTP-α), and PTPTY20 (PTPIB) are consistent with previous studies. PTPTY8 (HPTPe) is expressed at the highest levels in brain and testes and at a lower level in a variety of other tissues. Among the novel PTP genes, PTPTY43 was expressed at comparable levels in a wide variety of tissues. In contrast, PTPTY42 was highly restricted in its expression and was detected at low levels in RNA from liver, kidney, spleen, or fetal liver and at high levels in RNA from thymus. This pattern of expression was almost identical to the pattern of expression of Ly5/CD45 (PTPTY1). The remaining novel PTP, PTPTY35, was found at high levels in RNA from liver, kidney, and brain and at much lower levels in RNAs from other tissues.
Fig 3. Expression of PTP genes in murine hematopoietic cell lines. Poly(A)+ RNA was obtained from NIH 3T3 fibroblasts (1); DA-8, B cells (4); and NFS-112, B cells (5). Total RNA was obtained from DA-3, IL-3-dependent myeloid leukemia cells (2); DA-2, T cells (3); and BMS2F5, CSF-1-dependent macrophages (6). The RNAs were resolved on 1.2% agarose cells and transferred to nitrocellulose. The blots were subsequently hybridized with the indicated PCR-amplified cDNA clones. The size (kb) of the transcripts was determined relative to the migration of standards. Blots were also hybridized with probes for actin as a control.

Fig 4. Expression of PTP genes in murine tissues. Total RNA was obtained from the indicated tissues and resolved on 1.2% agarose cells. The RNAs were transferred to nitrocellulose and the blots were hybridized with probes to the indicated PCR-amplified cDNAs. The sizes (kb) of transcripts were determined relative to the migration of standards and are indicated. Blots were also rehybridized with probes for actin as a control.
DISCUSSION

Our studies were directed at identifying PTPs that are expressed in IL-3-dependent myeloid cells and that may contribute to IL-3-regulated growth control. To this end, we used a PCR amplification method that is based on the existence of conserved sequences within gene families. Comparable approaches have been used to identify novel PTKs as well as new members of other gene families. More recently comparable approaches have been used to identify PTP domains in cDNAs from sea squirts and from human liver. In our studies, the validity of the approach was shown by the isolation of cDNAs for two known PTP genes and for two genes that are likely to be the murine homologs of cloned human genes. It can be anticipated that the techniques described here will be useful for the identification of novel PTPs in other cell types.

PCR amplification of cDNAs from IL-3-dependent cells resulted in the identification of seven known or potential PTP genes. The number of PTPs that would be expected is not known. However, we have used similar PCR amplification techniques to identify the PTKs that are present in IL-3-dependent cells. These studies identified cDNAs for JAK1, JAK2, c-hck, c-lyn, c-fes, c-abl, insulin-like growth factor I receptor, and two novel receptor-like kinases (Yi and Ihle, unpublished data). The similarity in numbers suggests the possibility that each of these kinases has an associated phosphatase that has specificity for either the kinase itself or the substrates of the kinase.

Based on previous studies, it was anticipated that cDNA clones for Ly5/CD45/LCA and LRP/R-PTPα should be obtained from IL-3-dependent cells. However, it was not expected that murine homologs of PTP1B and HPTPe would be expressed in IL-3-dependent cells. The gene encoding HPTPe was initially cloned from a human placental cDNA library. Our studies show that the murine homolog is expressed at high levels in lymphoid and myeloid cells and is not expressed in fibroblasts. A comparable specificity was seen in Northern blots of RNAs from human myeloid and fibroblast cell lines (Yi, Cleveland, and Ihle, unpublished data). The hematopoietic specific pattern of expression of PTPY43 is also seen in Northern blots of murine tissues. Importantly, the pattern of expression of PTPY42 in murine tissues is identical to that of Ly5/CD45/LCA. The size of the PTPY42 transcript (2.6 kb) would suggest that the gene encodes a relatively small phosphatase and thus would not be expected to be a member of the membrane-associated phosphatases that have extracellular domains. Because of the hematopoietic specific pattern of expression of PTPY42, it will be important to obtain full-length cDNA clones and to assess the function of this novel potential PTP in hematopoietic cells.

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