Mild impairment of motor nerve repair in mice lacking PTP-BL tyrosine phosphatase activity

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Wansink, Derick G., Wilma Peters, Iris Schaafsma, Roger P. M. Sutmuller, Frank Oerlemans, Gosse J. Adema, Bé Wieringa, Catharina E. E. M. van der Zee, and Wiljan Hendriks. Mild impairment of motor nerve repair in mice lacking PTP-BL tyrosine phosphatase activity. Physiol Genomics 19: 50–60, 2004. First published June 29, 2004; doi:10.1152/physiolgenomics.00079.2004.—Mouse PTP-BL is a large, nontransmembrane protein tyrosine phosphatase of unclear physiological function that consists of a KIND domain, a FERM domain, five PDZ domains, and a COOH-terminal catalytic PTP domain. PTP-BL and its human ortholog PTP-BAS have been proposed to play a role in the regulation of microfilament dynamics, cytokinesis, apoptosis, and neurite outgrowth. To investigate the biological function of PTP-BL enzyme activity, we have generated mice that lack the PTP-BL PTP moiety. These PTP-BLΔP mice are viable and fertile and do not present overt morphological alterations. Although PTP-BL is expressed in most hematopoietic cell lineages, no alterations of thymocyte development in PTP-BLΔP mice could be detected. Sciatic nerve lesioning revealed that sensory nerve recovery is unaltered in these mice. In contrast, a very mild but significant impairment of motor nerve repair was observed. Our findings exclude an essential role for PTP-BL as a phosphotyrosine phosphatase and rather are in line with a role as scaffolding or anchoring molecule.

FERM domain; PDZ domain; gene targeting; signal transduction; sciatic nerve

M ost signaling events involving reversible protein tyrosine phosphorylation emanate at the cell cortex, and it should therefore come as no surprise that many protein tyrosine phosphatases (PTPs) are of the transmembrane receptor type (2). In addition to those, there is a subset of nonreceptor type PTPs, consisting of PTPH1, MEG1, PTPD1, PTPD2, and PTP-BAS in human, which is directed to the cell cortical area by virtue of a so-called FERM domain. The FERM domain is an acronym of “band 4.1, ezrin, radixin, and moesin” (9). These four proteins are capable of binding, in a regulated fashion, to transmembrane proteins and phospholipids with their NH2-terminal FERM domain and to microfilaments through their COOH-terminal actin binding domain, thereby providing a link between the cell membrane and the cytoskeleton (7). Some FERM domain-containing PTPs have in addition one (in PTPH1 and MEG1) or five (for PTP-BAS and its mouse ortholog PTP-BL) PDZ domains, small globular protein domains named after the first proteins in which they were identified: PSD95, DlgA, and ZO-1. PDZ domains can be found in a wide variety of proteins and are known to mediate protein-protein interactions by specifically recognizing COOH-terminal sequences of their targets, but some may also bind to internal peptide stretches (51). In this way, PDZ-containing proteins help to orchestrate the composition of protein complexes and thus contribute to the necessary hardware for signaling and adhesive processes (39). Recently, at the very NH2-terminus of PTP-BAS, a kinase noncatalytic C lobe domain (KIND) was identified (10), which may even further expand the anchoring and scaffolding potential of this PTP. Thus FERM and PDZ domain-containing PTPs all have the potency to be highly relevant components of cell cortical “signalosomes.”

Experimental evidence for the above hypothesis is sparse, however. PTPH1 has been shown to interact with three different proteins: 14-3-3β, vasolin-containing protein/p97/CDC48, and tumor necrosis factor α-convertase (TACE) (55–57). As a consequence, it is believed to play a role in TACE-dependent ectodomain release (57) and cell cycle progression (56). Expression studies in T cells also implicate PTPH1 in the reduction of antigen receptor signaling (25). Transfection experiments with MEG1 have pointed to inhibitory effects on cell proliferation and colony formation (24), and interaction studies revealed a potential role for MEG1 as a regulator and as a downstream signal transducer of glutamate receptors (28). For PTP-BAS/PTP-BL, potentially interacting proteins have been identified that point to a role in actin dynamics (12, 14, 20, 22, 35, 41, 50), and overexpression studies suggest regulatory roles in processes as diverse as NFκB (29, 36) and ephrin (37) signaling, cytokinesis (27), and Fas-mediated apoptosis (31, 42). Also, a gene trap insertion strategy resulted in mice that express a β-galactosidase-tagged NH2-terminal fragment of PTP-BL, and the observed staining pattern in peripheral nerves and spinal ganglia led to the suggestion that this large intracellular PTP might play a role in neurite outgrowth (44).

The enzymatic activity of PTP-BL’s PTP domain, as demonstrated in vitro using its potential partner protein RIL (“reversion-induced LIM” protein) as substrate (12), needs to be tightly regulated since ectopic expression in COS-1 cells of the PTP domain alone, i.e., without its proper targeting signals, induces morphological abnormalities (15). As a means to study the relevance and biological function of PTP-BL-mediated dephosphorylation of phosphotyrosines at the cell cortex, we generated mice deficient in PTP-BL phosphatase activity through gene targeting in mouse embryonic stem (ES) cells. Resulting mice, which express a COOH-terminally truncated protein (PTP-BLΔP) and lack the wild-type (WT) enzyme, are
viable and fertile and, after backcrossing to a C57BL/6 background, do not present evident growth defects. Furthermore, no gross anatomical alterations have been observed, and lymphoid development was unimpaired. Sciatic nerve lesional studies, however, point to a mild delay in motor neuron outgrowth as a result of the targeted mutation. This finding is in line with a role for PTP-BL in neurite outgrowth.

MATERIALS AND METHODS

PTP-BL targeting vector construction and mouse production. Mouse 129 genomic clones were identified in a cosmid library (kindly provided by M. Hofker) according to standard procedures. A 4.3-kbp EcoRI-BamHI fragment, containing exon 41 of the PTP-BL gene (Ptpn13, LocusID 19249), was subcloned in front of a neomycin phosphotransferase expression cassette (neo) (48). Downstream of the resistance cassette, a 3.4-kbp EcoRI fragment was introduced that harbors the last exon (exon 47) of the PTP-BL gene. Prior to electroporation, the targeting construct was linearized using a unique SalI site. Targeting in E14 ES cells followed by subsequent injection into C57BL/6 blastocysts and breeding with chimeric males was done as described (49).

Mice were kept at the Central Animal Facility of the University of Nijmegen in a standard room with a 12 h/day/night rhythm of 06:00/18:00 h at a temperature of 21 °C and a humidity of 50–60%. Mice were housed in Macrolon cages and fed ad libitum. Experiments were performed on F3 mice with a 129/Ola × C57BL/6 hybrid genetic background and on F9 animals that resulted from seven successive backcrosses onto C57BL/6 background and subsequent intercross of resulting heterozygotes. All procedures involving animals were approved by the Animal Care Committee of the University Medical Center St. Radboud (Nijmegen, The Netherlands) and conformed to the Dutch Council for Animal Care and the NIH guidelines.

Genotyping cells and mice. ES cell genomic DNA was extracted and analyzed by Southern blot analysis as described (34). Screening was performed using a 300-bp HindIII genomic fragment as a 3′ probe on BamHI digested ES cell DNA, a 600-bp HindIII fragment as a 5′ probe on EcoRV digests, and a 1.1-kbp Khol fragment from the neo cassette to rule out additional integrations of the vector. DNA obtained from toe clips or tail biopsies was screened by Southern blot analysis or by PCR using primers BL-sense 5′-TGACACCTCAGGCCAGCTGTTGAG-3′, BL-antisense 5′-CAGTAGGTCTTGAGAAATTTG-3′, and Neo-3′-forward 5′-CTATCGCTTCTTCTGCAGATT-3′.

RNA analysis. Total RNA from kidney, stomach, brain, and liver was isolated using the LiCl/urea-phenol-chloroform extraction method (3). For Northern blot analysis, 20 μg RNA of each sample was fractionated on a 1% (wt/vol) agarose/2.2 M formaldehyde gel and transferred to Hybond-N membrane (Amersham). Blots were probed with mouse PTP-BL cDNA fragments (26) or a 1.1-kbp HI fragment, containing exon 41 of the PTP-BL gene (Ptpn13, LocusID 19249), with pre-immune serum, and analyzed by Southern blot analysis as described (43). Screening was performed using a 300-bp HindIII genomic fragment as a 3′ probe on BamHI digested ES cell DNA, a 600-bp HindIII fragment as a 5′ probe on EcoRV digests, and a 1.1-kbp Khol fragment from the neo cassette to rule out additional integrations of the vector. DNA obtained from toe clips or tail biopsies was screened by Southern blot analysis or by PCR using primers BL-sense 5′-TGACACCTCAGGCCAGCTGTTGAG-3′, BL-antisense 5′-CAGTAGGTCTTGAGAAATTTG-3′, and Neo-3′-forward 5′-CTATCGCTTCTTCTGCAGATT-3′.

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Antibodies. To obtain antibodies directed against an NH2-terminal part of PTP-BL (amino acids 50–443), the corresponding cDNA fragment was cloned in-frame in the pGEX-2T vector (Amersham Biosciences UK Limited, Buckinghamshire, UK) to produce a recombinant GST-Bl-N fusion protein in Escherichia coli. The recombinant protein was purified using glutathione-Sepharose CL4B beads (Amersham) and analyzed on 10% polyacrylamide gels. Polyclonal αBl-N antiserum was generated by immunizing rabbits with GST-Bl-N fusion protein according to established protocols. The rabbit polyclonal αBl-N antiserum (recognizing the phosphatase domain) has been described previously (12).

Western blot analysis. Protein lysates of several tissues of WT, PTP-BL+/ΔP, PTP-BLΔP/ΔP mice were prepared by homogenization in lysis buffer [20 mM HEPES, pH 7.5, 2 mM EGTA, 10% glycerol, 1 mM PMSF, 10 mM NaF, 1 mM orthovanadate, and 1 protease inhibitor cocktail tablet (Roche Diagnostics, Mannheim, Germany) per 50 ml] followed by adding detergents to a final concentration of 0.5% (wt/vol) NP-40 and 0.5% (wt/vol) SDS. Whole cell homogenates were incubated on ice for 30 min, after which an equal volume of Laemmli sample buffer was added, followed by incubation for 15 min at 90°C. Protein concentration of the resulting samples was determined according to a modified Lowry procedure (38). Twenty micrograms of protein of each tissue was loaded per lane on a 6% polyacrylamide gel and after electrophoresis transferred to nitrocellulose membrane by Western blotting. Blots were processed and incubated with αBl-N or αBl-PTP followed by chemiluminescence detection using standard procedures.

Lymphocyte preparations and flow cytometry. Single-cell suspensions were prepared by gently pressing lymphoid organs from 6- to 8-wk-old WT and PTP-BLΔP/ΔP mice between frosted glass slides. Total lymphocytes were purified by filtration through Nitex nylon mesh ( pore size, 53 μm). Cells (106) were stained with anti-CD3-PE, anti-CD4-APC, anti-CD8-FITC, anti-CD11c-APC, anti-CD25-FITC, (BD Biosciences), anti-GR-1, anti-F4/80 (Caltag Laboratories, Burlingame, CA), and anti-B220 (6B2 hybridoma supernatant) antibodies according to standard procedures. Flow cytometry was performed on a BD Biosciences FACSCalibur. Propidium iodide-negative cells were analyzed using CELLQuest software.

Immunohistochemistry. Cryosections of mouse C57BL/6 tissues snap-frozen in liquid nitrogen were cut (7 μm) and mounted on SuperFrost/Plus slides (Menzel-Glazer, Braunschweig, Germany). After drying, sections were fixed in 1% formaldehyde in 0.1 M phosphate buffer (PB) for 10 min. Sections were processed and incubated with pre-immune serum, αBl-N, or αBl-PTP according to standard procedures and embedded in Mowiol.

Histological analysis. Various tissues from adult male and female mice were dissected, fixed in buffered formaldehyde, dehydrated, and embedded in paraffin. Sections of 6-μm thickness were stained with hematoxylin-eosin according to standard histological procedures. Brains were analyzed using parasagittal and coronal sections stained with cresyl violet.

Unilateral sciatic nerve crush lesion and functional recovery. The sciatic nerve of the right hind paw in WT and PTP-BLΔP/ΔP mice was subjected to a crush lesion as described previously (16, 46, 47). In short, mice were anesthetized, and the sciatic nerve was carefully exposed. With forceps, the nerve was crushed for 60 s at the sciatic notch point immediately distal from where it emerges from under the gluteus maximus muscle. The skin was sutured, and mice were returned to their home cage. Return of sensory function was determined by applying a range of small electric currents (0.1, 0.3, and 0.5 mA) to the foot sole of the mice. Absence of the foot-withdrawal reflex at 0.5 mA, current, for three consecutive days, were considered to be recovered. The withdrawal reflex was measured at postlesion day 3 and daily from day 10 onward until full recovery.

The recovery of motor function following sciatic nerve crush lesion was monitored through analysis of the individual mouse free-walking pattern. The walking test method was originally described for rats (19), and calculations were modified by De Koning and Gispen (17; see also Refs. 33 and 52). The progress of motor function recovery in the sciatic nerve was calculated using eight footprint parameters (distances in mm) and a correction factor (applicable to mice as well) according to the following formula:

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SFI = [(EOTO - NTOF) / NTOF + (NPL - EPL)] / EPL \\
+ [(ETS - NTS) / NTS + (EIT - NIT) / NIT] \times 46
\]

where SFI is sciatic functional index (%); NTOF is normal to opposite foot; ETOF is experimental to opposite foot; NPL is normal print
length; EPL is experimental print length; NTS is normal toe spreading; ETS is experimental toe spreading; NIT is normal inner toe spreading; and EIT is experimental inner toe spreading. This formula is derived from the postlesion day 3; ETS and EIT are both set at 2 mm) and an SFI between −10% and +10% for nonlesioned control mice and when full recovery of motor function is obtained.

In the test procedure each mouse was allowed to acclimate to the experimental environment by once letting the animal walk through an inclining (10°) alley (40 × 3.5 cm) that leads into a dark box. Then, a strip of photographic paper (Kodak, Polymax II RC semi-matt) was placed on the bottom of the alley, and, after dipping the animal’s hind feet in photographic paper developer fluid (Kodak, Polymax RT), the animal was again placed at the beginning of the alley to let it walk into the dark box. Subsequently, after allowing the photographic strip to dry, mouse footprint parameters (indicated above) were measured. In this way, motor function recovery was determined at day 3 and then every second day starting from day 8 postlesion.

Neurofilament-200 immunostaining and quantification analysis. Following transcardial perfusion of WT and PTP-BLΔP/ΔP mice, sciatic nerve dissection, and cryostat sectioning, a neurofilament (NF)-200 immunostaining of 8-μm-thick peripheral nerve sections was performed as described (47), using rabbit anti-NF-200 (1:1,000; Sigma Chemical, St. Louis, MO). For the quantitative analysis, digital images of the sciatic nerve sections (on coded slides) were collected using a Dialux 20 microscope (Leitz) connected to a video camera attached to a PC image-analysis system. The number of NF-200-positive nerve sprouts was counted in three nerve sections (8 μm thick, 72 μm apart) at 1, 3, and 5 mm distal to the crush site at 4 and 7 days post crush lesion. Newly outgrowing axonal sprouts, as revealed by NF-200 antibody/3-amino-9-ethylcarbazole (AEC) immunostaining, appeared under the microscope as relatively large, medium-sized, or small red dots. Counting was performed using an ocular grid with 10 squares, which covered and represented therefore a total sciatic nerve area of 6,250 μm². The total axon number per 6,250 μm² was calculated from counts in three to six sections per distance, per mouse, and per genotype group.

Statistics. Data obtained from WT and PTP-BLΔP/ΔP mice are presented as means ± SE. All data measurements were obtained blind as to genotype and subsequently analyzed using the appropriate statistical tools (one-way ANOVA, ANOVA repeated measures, Student’s t-test, paired t-test; SPSS 10.0 statistics software). Statistical significance was set at P < 0.05.

RESULTS AND DISCUSSION

Targeted deletion of PTP-BL phosphatase domain gene segment. To study the function of PTP-BL as a tyrosine-specific phosphatase in the cell cortical region, we used gene targeting by homologous recombination in ES cells to produce PTP-BLΔP/ΔP mice that would lack the PTP-BL tyrosine phosphatase moiety. Using a replacement-type targeting vector, exons 42–46 of the mPTP-BL gene, Ptpn13, encoding the phosphatase domain of PTP-BL, were replaced by the neo selection cassette (Fig. 1A). Southern blot analysis using a 3’ diagnostic probe (Fig. 1B), and a 5’ diagnostic probe (Fig. 1C) was used to confirm proper recombination. Furthermore, ES lines were tested for the presence of only one copy of the neo cassette (data not shown). Successfully targeted ES clones

Fig. 1. Disruption of the PTP-BL gene by homologous recombination. A: the PTP-BL protein structure showing the various domains (KIND, FERM, PDZ I–V, and PTP) is depicted on top. A schematic diagram of the relevant part of the mouse Ptpn13 locus and the targeting strategy is shown at the bottom. Exons are indicated as small black boxes. In the targeting construct, the fragments homologous to PTP-BL genomic segments are termed 5’- and 3’-arm, respectively, and the white box termed “neo” symbolizes the neomycin phosphotransferase selection cassette. Small black bars below the wild-type (WT) allele, designated 5’p and 3’p, respectively, indicate genomic segments used as 5’ and 3’ diagnostic probes. Arrows indicate location of primers used for genotyping by PCR. B. BamHI; E, EcoRI; Ev, EcoRV. B: Southern blot analysis of BamHI-digested genomic DNA from WT (+/+) and correctly targeted embryonic stem (ES) cells (+ΔP) using the 3’ diagnostic probe, which detects the mutant 4.5-kbp BamHI fragment and the WT 10.6-kbp BamHI fragment. Size markers (kb) are indicated on the left. C: Southern blot analysis of EcoRV-digested genomic DNA from WT and correctly targeted ES cells hybridized with the 5’ diagnostic probe. Signals obtained correspond to a WT >30-kbp EcoRV fragment and an ~18-kbp mutant EcoRV fragment. D: Southern blot analysis of BamHI-digested genomic DNA from WT, heterozygous (+/ΔP), and homozygous (ΔP/ΔP) PTP-BL mice using the 3’ diagnostic probe. E: PCR analysis on genomic DNA from +/+; +/ΔP, and ΔP/ΔP mice demonstrates only WT alleles (wt) in +/+ mice and targeted alleles (tg) in ΔP/ΔP mice, whereas +/ΔP animals contain one allele of each type. Fragment sizes are ~500 bp for the WT PCR and ~400 bp for the targeted allele.
were used for blastocyst injection followed by breeding with chimeric males. F1 offspring carrying the PTP-BLΔP mutation were mated with C57BL/6 mice to rapidly expand the population. Presence of the PTP-BLΔP allele was confirmed in offspring from F2 heterozygotes by Southern blotting (Fig. 1D) and PCR analysis of genomic DNA (Fig. 1E). The PTP-BLΔP allele was identified in 96 heterozygous (PTP-BL+ΔP, 49%) and 49 homozygous (PTP-BLΔPΔP, 25%) mice out of a total of 195 animals tested. Thus the mutant allele segregated according to Mendelian law. Subsequent breeding steps demonstrated unaltered fertility and litter sizes for heterozygous and homozygous mutant animals compared with WT littersmates, indicating that removal of the PTP-BL PTP domain did not impair essential steps in development and reproduction.

The PTP-BLΔP allele produces truncated mRNAs. PTP-BL is expressed in many epithelial cell types, in brain, and in peripheral nerves and spinal ganglia (26, 44, 50). To investigate consequences of PTP-BL gene targeting at the RNA level, total RNA was isolated from several mouse tissues and analyzed by Northern blot analysis (Fig. 2). PTP-BL is not expressed in liver, which was therefore taken as a negative control. Use of the full-length PTP-BL cDNA as a probe on WT RNA revealed several different transcript isoforms that are 8–9 kb in size and most likely result from alternative splicing (4, 8, 32, 42). In PTP-BLΔPΔP RNA samples, shorter versions of these transcripts, around 7–8 kb in size, result from transcription of the PTP-BLΔPΔP allele. RNA isolates from heterozygous tissues demonstrate that the WT and the PTP-BLΔPΔP mRNAs are present in comparable amounts (Fig. 2A). As expected, using a mouse PTP-BL cDNA probe consisting of the PTP-encoding part, we could detect no mRNAs in PTP-BLΔPΔP mice (Fig. 2B).

To investigate the nature of the transcripts resulting from the PTP-BLΔP allele in more detail, we performed RT-PCR experiments on PTP-BLΔPΔP-derived RNA and cloned and sequenced the obtained fragment. This revealed that PTP-BLΔP mRNAs lack the sequence parts corresponding to exons 41–46 of the PTP-BL gene. Evidently, genomic deletion of Ptpn13 exons 42–46 resulted in PTP-BLΔP primary transcripts in which the exon 40 splice donor site is used in conjunction with the splice acceptor site of the very last Ptpn13 exon, exon 47. As a result, PTP-BLΔPΔP mRNA variants are 961 bases shorter than their WT counterparts and encode PTP-BL mutant forms that, from amino acid residue 2100 onward, lack the COOH terminus containing the catalytic PTP protein part. Instead, such PTP-BLΔPΔP proteins would have a 14 amino acids long, rather serine-rich tail (-RVSMSSAIKSSSMS) due to a change in the reading frame used in exon 47-derived sequences (depicted in Fig. 2C). Database searches demonstrated that this artificially created COOH-terminal sequence is not present in any other protein reported in human or mouse.

Truncated PTP-BL protein is overrepresented in PTP-BL+ΔP mice. To determine whether PTP-BLΔPΔP transcripts indeed give rise to truncated PTP-BL protein forms, we performed Western blot analyses of protein lysates from several mouse tissues using polyclonal antiserum directed against two different parts of PTP-BL (i.e., the N-terminal segment and the catalytic PTP segment, respectively). In WT animals two major PTP-BL protein isoforms around 270 kDa in size were detected by the two sera in brain, kidney, lung, and testis lysates, but not in liver, as expected (Fig. 3A). In tissue lysates from homozygous PTP-BLΔPΔP mice, no specific immunoreactivity toward the αBL-PTP antiserum was observed (Fig.

![Image](http://physiolgenomics.physiology.org/)
Fig. 3. Truncated, PTP-BLΔP protein is more stable than WT PTP-BL. A: equal protein amounts from lysates of WT (+/+), heterozygous (+/ΔP), and homozygous PTP-BLΔPΔP (ΔP/ΔP) mouse tissues were separated by SDS-PAGE, blotted onto PVDF membranes, and immunostained with αBL-N antiserum. Two WT PTP-BL isoforms, probably different splice isoforms (arrowheads), can be detected in brain, kidney, lung, and testis samples of WT and heterozygous mice. PTP-BL is not expressed in liver. In PTP-BLΔPΔP lysates stable truncated, PTP-BLΔP products are clearly detectable. Signal intensity of the various bands in heterozygous material revealed that PTP-BLΔP is two to four times more abundant than WT protein. B: the αBL-PTP antiserum did not yield a specific signal in PTP-BLΔPΔP lysates, indicating that the corresponding epitope was absent. Asterisks indicate nonspecific signals, unrelated to PTP-BL, observed in brain, lung, and testis. When using αBL-N antiserum on brain, lung, and testis samples, the mobility of one aspecific band unfortunately coincides with that of the larger PTP-BLΔP isoform. A size marker is indicated on the left (kDa).

3B), but indeed two truncated forms of PTP-BL were detected using the αBL-N antiserum (Fig. 3A).

Remarkably, in the PTP-BL+/ΔP samples the amount of PTP-BLΔP proteins exceeds that of WT isoforms. Signal quantitation by densitometry revealed a two- to fourfold overexpression of PTP-BLΔP isoforms compared with the full-length PTP-BL expression level in WT animals (Fig. 3A). This suggests that the truncated, phosphatase-dead protein is more stable, which may reflect the action of possible regulatory mechanisms responding to the loss of PTP-BL catalytic activity.

To examine the phosphorylation status of PTP-BL/PTP-BLΔP and the PTP-BL substrate RIL in PTP-BLΔPΔP and WT mice, lysates from brain, lung, kidney, and testis were used in immunoprecipitation experiments applying αBL-N, αBL-PTP, and αRIL antisera. Immunoprecipitates were tested on immunoblots using different phosphotyrosine-specific antibodies, but no change in tyrosine phosphorylation levels was observed (data not shown). We also investigated in these tissue lysates whether proteins that colocalize with (α-actinin, ezrin) or can bind to PTP-BL (PARG, RIL) have altered expression levels in PTP-BLΔΔP mice, but again no effect of PTP-BL phosphatase deficiency was apparent (data not shown).

WT PTP-BL protein is localized at the apical side of polarized epithelial cells in stomach and lung tissue (12). Deletion of the PTP-BL PTP domain did not alter this subcellular localization (Fig. 4), which is in line with previous observations pointing to the FERM domain in PTP-BL as being responsible for cortical targeting (6, 15).

Histological analysis of PTP-BLΔPΔP mice. We performed an extensive histological survey to look for possible consequences of the absence of enzymatically active PTP-BL at the tissue and cellular level. A wide variety of tissues, including brain, kidney, liver, lung, stomach, intestinal tract, testis, spleen, and thymus, were isolated, sectioned, and stained with hematoxylin-eosin. In addition, serial parasagittal and coronal sections of brain tissue were stained with cresyl violet and analyzed for abnormalities. No obvious differences between adult PTP-BLΔPΔP and WT mice were discerned (data not shown).

Increased body weight in the initial cohort of PTP-BLΔPΔP male mice. Mice lacking the PTP-BL PTP domain behaved normally and appeared healthy. Over time, however, we noted that male, but not female, PTP-BLΔPΔP mice, which resulted from intercrosses of heterozygous F2 animals, developed a mild obese phenotype (Fig. 5, A and C). First signs could be observed around 4 mo of age, and at the age of 15 mo the average body weight of PTP-BLΔPΔP males (~42 g) differed significantly (P < 0.002) from that of WT male littermates (~33 g). PTP-BLΔ+/ΔP males showed intermediate values but were also significantly heavier than WT (~37 g, P < 0.03; Fig. 5A). Macroscopic and histologic examination revealed that the
increase was due to an increase of white adipose tissue and a concomitant general enlargement of other tissues, especially liver.

Subsequently, PTP-BL^{+/ΔP} mice which had been backcrossed to the C57BL/6 genetic background over seven successive generations became available, and body weight measurements were repeated on littermates resulting from intercrosses between these heterozygous F9 animals. This time no differences could be detected, not even when comparing 2-yr-old animals (Fig. 5, B and D). Furthermore, introduction of a high-fat diet resulted in a comparable rate of body weight increase for the different genotype groups (data not shown).

Normal lymphoid development in PTP-BL^{ΔP/ΔP} mice. The human ortholog of PTP-BL, PTP-BAS, was originally identified in basophils (32) and subsequently shown to be widely expressed in lymphoid cells (21). Furthermore, a direct and functional interaction between PTP-BAS and the human Fas death receptor has been reported in cell models (42), although this could not be confirmed in mouse (13). Therefore, to trace a possible distortion on lymphoid developmental processes, we analyzed the lymphocyte composition of thymus, spleen, and lymph nodes using flow cytometry (Table 1). Mature T cells are CD3 positive and can be classified according to the expression of CD4 and CD8 cell surface markers. Most immature T cells do not express CD4 and CD8 and thus will be double negatives. Maturation initially results in CD4/CD8 double-positive cells, representing the majority of thymocytes, which then gives rise through the mechanisms of positive and negative selection to mature single-positive cells by losing either

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**Fig. 5.** Gradual increase in body weight in F3 male PTP-BL^{ΔP/ΔP} mice. Body weight of WT (open squares), heterozygous (open triangles), and homozygous PTP-BL^{ΔP/ΔP} (solid circles) mice was determined at regular intervals. Average values for each genotype at each time point are plotted, and bars indicate standard errors of the mean. These data were used for curve-fitting analysis using nonlinear regression (+/+, dotted curve; ΔP, dashed curve; ΔP/ΔP, solid curve). Two different cohorts of mice were used, as follows. 1) F3 generation males (A) and females (C) resulting from intercrosses of F2 PTP-BL^{+/ΔP} animals (on average a 129/C57BL/6 genetic background ratio of 1:3); n = 22, 40, and 28 for ++/+ , +/ΔP, and ΔP/ΔP male mice, respectively; n = 22, 25, and 13 for ++/+ , +/ΔP, and ΔP/ΔP female mice, respectively. 2) F9 generation males (B) and females (D) obtained after seven successive backcrosses onto C57BL/6; n = 28, 34, and 18 for ++/+ , +/ΔP, and ΔP/ΔP male mice, respectively; n = 26, 32, and 19 for ++/+ , +/ΔP, and ΔP/ΔP female mice, respectively. Body weights of F3 male mice are significantly different between ΔP/ΔP and ++/+ animals (P < 0.002, Wilcoxon), and between +/ΔP and ++/+ mice (P < 0.03). No significant difference was found when +/ΔP male mice were compared with ΔP/ΔP males (P = 0.56). In F3 female mice also no significant difference in body weight was observed between the genotype groups. Clearly, the almost completely overlapping curves for the F9 genotype cohorts (B and D) demonstrate equal body weights within both sex groups.
CD4 or CD8 markers. CD3 is poorly expressed by immature thymocytes but highly expressed by mature T cells. Single-positive T cells then exit the thymus and seed peripheral lymphoid organs such as lymph nodes and the spleen. We checked for the relative contribution of the different thymocyte subsets in the various lymphoid organs (Table 1). In addition, the B lymphocyte population was monitored using a CD45 isotype-specific antibody, B220. The Gr-1 antibody allowed detection of cells from the myeloid lineage, like monocytes and granulocytes. The F4/80 antibody was used to analyze macrophages. Antigen-presenting cells (APCs) were monitored using anti-CD11c. Finally, regulatory T cells were monitored by the expression of CD4 and CD25 (40). All PTP-BlAPDP lymphoid organs examined had mature T cells in proportions that were identical to that of WT specimen. Likewise, the subpopulations of thymocytes, the CD4+CD25+ Treg cells, the percentages of B cells, and the amounts of Gr-1-, F4/80-, or CD11c-positive cells were unchanged. Finally, the sizes of lymphoid organs were comparable between PTP-BlAPDP and WT animals, and normal numbers of cells could be isolated. In conclusion, no obvious differences in lymphoid tissue composition were detected between samples from PTP-BlAPDP and WT animals and WT controls.

Delayed motor, but not sensory, function recovery following sciatic nerve crush in PTP-BlAPDP mice. The expression of PTP-Bl in the developing peripheral nervous system (44) prompted us to investigate the localization of PTP-Bl in the adult animal and the role of PTP-Bl in nerve regeneration. Immunostaining of transversal sections of WT spinal cord and dorsal root ganglia with rabbit antiserum aBl-PTP, which recognizes the phosphatase domain, demonstrated that PTP-Bl is expressed in small and large dorsal root ganglia sensory neurons (Fig. 6A) and in most spinal cord motor neurons (Fig. 6B). Similar sections from PTP-BlAPDP mice served as specificity controls (not shown). The presence of PTP-Bl in dorsal root ganglia and spinal cord motor neurons is in agreement with earlier findings (44).

To evaluate the effect of the PTP-BlAPD mutation on peripheral nerve regeneration, a unilateral crush lesion of the sciatic nerve was applied. Gradual recovery of the sensory function of the sciatic nerve was assessed daily by applying a small current stimulus to the foot sole and scoring the subsequent occurrence or absence of the foot-withdrawal reflex in WT (n = 9) and PTP-BlAPDP (n = 10) mice (Fig. 7A, showing data regarding 0.1 mA). In WT mice, the sensory function response of the nonlesioned sciatic nerve in the contralateral paw showed a fast foot-withdrawal reflex upon stimulation with a 0.1-mA current stimulus. This response was equally fast in the nonlesioned contralateral paw of PTP-BlAPDP mice. First signs of sensory recovery in the lesioned hind paw of WT animals appeared as early as postlesion day 14, and all animals in the WT group were recovered by postlesion day 19 (average recovery, 17.2 ± 0.5 days; Fig. 7A). The first positive sensory recovery in the PTP-BlAPDP group was scored at postlesion day 15, and all animals were finally recovered by day 24. Average recovery time for the PTP-BlAPDP mice was 17.9 ± 0.9 days, which was not significantly different from that of the WT group.

In a larger group of animals, which also included the above-mentioned mice, the gradual recovery of motor function following the sciatic nerve crush lesion was assessed by monitoring the animal’s gait in a walking alley, measuring various footprint parameters and using these to calculate the sciatic function index (SFI, see Fig. 7B). Before the lesion, WT (n = 19) and PTP-BlAPDP (n = 23) mice showed identical gait, indicating normal motor function with regard to walking and the use of leg, paw, and toe muscles. At 3 days following sciatic nerve crush, an SFI value between 80 and 100% established the completeness of the crush lesion for all animals. The first signs of motor function recovery in WT mice became apparent at postlesion days 8 and 10 (with SFI, −76 ± 4.1% and −75 ± 6.4%, respectively; Fig. 7B). Subsequently, the motor function continued to improve over time, showing an SFI of −40 ± 5.2% at 2 wk postlesion. Full recovery was reached at postlesion day 23 (SFI, −8 ± 2.4%; Fig. 7B), which was in agreement with previous findings (33, 47).

In PTP-BlAPDP mice the index was −77 ± 3.4% and −76 ± 2.1% at postlesion days 8 and 10, respectively (Fig. 7B). At 2 wk postlesion the average SFI was still −55 ± 4.3%.

Table 1. Lymphocyte populations in PTP-BlAPDP mice

<table>
<thead>
<tr>
<th></th>
<th>Thymus</th>
<th>Spleen</th>
<th>Lymph Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>PTP-BlAPDP</td>
<td>WT</td>
</tr>
<tr>
<td>Total cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F4/80</td>
<td>0.0</td>
<td>0.0</td>
<td>6.5 (2.4)</td>
</tr>
<tr>
<td>Gr-1</td>
<td>0.8</td>
<td>1.0</td>
<td>6.2 (0.9)</td>
</tr>
<tr>
<td>B220</td>
<td>0.7</td>
<td>0.8</td>
<td>38.6 (6.5)</td>
</tr>
<tr>
<td>CD11c</td>
<td>0.2</td>
<td>0.5</td>
<td>2.4 (0.3)</td>
</tr>
<tr>
<td>CD3</td>
<td>18.9</td>
<td>19.7</td>
<td>14.9 (3.6)</td>
</tr>
<tr>
<td>CD8</td>
<td>84.6</td>
<td>82.5</td>
<td>4.8 (0.9)</td>
</tr>
<tr>
<td>CD4</td>
<td>90.4</td>
<td>84.3</td>
<td>9.5 (1.9)</td>
</tr>
<tr>
<td>CD4CD8</td>
<td>84.0</td>
<td>80.8</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>CD4CD8</td>
<td>0.8</td>
<td>1.0</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>CD3+ cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>42.3</td>
<td>45.1</td>
<td>61.2 (2.8)</td>
</tr>
<tr>
<td>CD4CD4</td>
<td>40.8</td>
<td>40.6</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>CD4CD4</td>
<td>3.3</td>
<td>2.9</td>
<td>13.9 (2.6)</td>
</tr>
<tr>
<td>CD8</td>
<td>13.6</td>
<td>11.4</td>
<td>23.6 (3.0)</td>
</tr>
</tbody>
</table>

Values are in percent and are means, with standard deviations in parentheses. Analysis of the immune compartment of PTP-BlAPDP mice and littermate controls (age and gender matched, six animals per group) were used for flow cytometric analysis of immune cells. Single cell suspensions of thymus and lymph nodes of each group were pooled for cell number reason. Spleen samples were analyzed individually. No significant differences between PTP-BlAPDP mice and wild-type (WT) controls were observed. The representative result of one of two experiments is shown.
At days 23–25 in the fourth week, the SFI value remained at \(-16 \pm 4\%\), indicating that not all PTP-BL\(^{ΔPΔP}\) animals had reached complete recovery (Fig. 7B). By week 5, all animals were recovered (not shown). When statistically evaluated over the entire period from postlesion days 8–25, the motor function recovery in PTP-BL\(^{ΔPΔP}\) mice was significantly slower than in WT animals \([\text{ANOVA repeated measures, } F(1,40) = 4.59, P = 0.038}\].

**Diminished branching of regenerating axons in PTP-BL\(^{ΔPΔP}\) mice.** Subsequently, histological examination of the sciatic nerves was performed to determine the effect of the PTP-BL\(^{ΔPΔP}\) mutation on the number of peripheral nerve axons before and after crush lesion. First, NF-200 immunostaining of nonlesioned WT (Fig. 6C) and PTP-BL\(^{ΔPΔP}\) (Fig. 6D) sciatic nerves showed a similar staining pattern, with NF-200-positive axons appearing as small, medium-sized, or large red dots (Fig. 6, C and D). The average number of NF-200-positive axons per 6,250 \(\mu\text{m}^2\) sciatic nerve area in WT (154 ± 12, \(n = 8\)) and PTP-BL\(^{ΔPΔP}\) (168 ± 13, \(n = 9\)) mice was not significantly different, indicating that PTP-BL deficiency did not affect peripheral nerve development. Second, during the regeneration of the sciatic nerve, at 4 days following the crush lesion and at 5 mm distal to the crush site, WT (Fig. 6E) and PTP-BL\(^{ΔPΔP}\) (Fig. 6F) mice demonstrated NF-200-positive staining of newly formed axonal sprouts. Quantitative analysis involved counting of the number of NF-200-positive axonal sprouts at 4 days (Fig. 6G; \(n = 4\) per genotype group) and 7 days (Fig. 6H; \(n = 4\) per genotype group) post crush lesion, at 1, 3, and 5 mm distal to the crush site, and in three to six nerve sections per distance for each mouse. At 4 days postlesion, the average
distances were not significantly different between WT and
the gradual recovery of motor function was measured using the
PTP-BL functional index based on comparison of footprint parameters of the regen-
– 3 mm distance is no longer present in WT animals (Fig. 6
cant delay in motor function recovery of the
small but significant impairment of functional motor nerve repair in
PTP-BLΔpΔP mice.

Conclusions. The mutation introduced in the Ptpm13 gene results in the production of truncated, protein tyrosine phos-
phatase activity-deficient protein, PTP-BLΔp, which is more stable than full-length PTP-BL. The resulting overrepresentation
of anchoring and scaffolding modules, e.g., the KIND, FERM, and PDZ domains, in the cell cortical area may in itself have bearing on the composition and activity of protein complexes
involved in subcellular signaling and cell adhesion. Furthermore, the sheer reduction in tyrosine-specific phosphatase
capacity in the cell cortical area could well impair signaling
pathways during growth and development. Nevertheless, homozygous PTP-BL phosphatase-deficient mice are normal
in their overall morphology, behavior, fertility, and life span.
Also, lymphoid development patterns in the various genotype
groups were indistinguishable. Lack of abnormalities in stan-
dard gross pathology has also been reported for homozygous
PTP-BLΔp mice (44) that carry a gene trap insertion vector in
Ptpm13 intron 22, but upon careful examination these mice
were found to contain normal levels of WT PTP-BL in addition to the PTP-BL/β-galactosidase fusion protein (D. G. Wansink,
W. Hendriks, T. Thomas, and P. Gruss; unpublished data). The
insertion of a gene trap construct into a locus does not always
cause a reduction in the levels of WT mRNA produced at this
locus; for example, normal WT mRNA levels have been
reported in mice homozygous for a gene trap insertion into the
MAP4 locus (53).

Initially, we noted the development of an obesity syndrome
milder than the tubby obesity condition (11) for male but not
female PTP-BLΔpΔp mice. Sexual dimorphism is not uncom-
mon in obesity phenotypes (11), and the growth curve of
heterozygous PTP-BLΔpΔP males even pointed to a dominant
inheritance (Fig. 5A). However, a 129/Ola-derived modifier
locus obviously confounded these initial body weight analyses,
because subsequent backcrosses to the C57BL/6 genetic back-
ground completely eliminated the abnormalities in size and
weight of male mutant mice.

Upon performing peripheral nerve lesion studies, however,
we observed a small but significant impairment of sciatic motor
nerve function recovery in PTP-BLΔpΔP animals. The under-
lying mechanism remains as yet elusive, although subtle alter-
tations in guidance cues via ephrin/EphB signaling and/or
survival cues via p75 NTR may contribute (29, 37). Presum-
ably, under normal conditions in PTP-BLΔpΔp mice, other
 tyrosine-specific phosphatases can effectively compensate for the
dephosphorylation task of PTP-BL, or, alternatively, the
PTP-BLΔp protein has no detrimental effects. Following sci-
atic nerve injury, however, cellular processes apparently are
impaired in motor neurons compared with sensory cells, which
may reflect differential expression of certain PTPs in these cell
types as has been reported for the cell adhesion molecule-like
PTP LAR (54). Still, being the largest mammalian intracellular
PTP, PTP-BL contains protein stretches, such as the KIND
domain, that are not present in any other family member.
Furthermore, it has four more PDZ domains than comparable mammalian PTPs (i.e., PTPH1 and MEG1).

It is tempting to speculate that the main physiological contribution of PTP-BL to the functioning of cells and tissues is actually reflected by its potency to orchestrate the composition and dynamics of large protein machines through its many protein interaction modules rather than by its enzymatic activity. One could even envision that PTP-BL directly recruits other PTPs into signaling complexes, which then may overcome the PTP-BLΔP enzyme mutation. Inter-PTP interactions have been noted for other family members (e.g., Refs. 5 and 23), and RPTPθ, for example, possesses a COOH-terminal PDZ binding domain (1) that may well represent a target for PTP-BL PDZ domains. Characterization of mutant mice completely devoid of any of the noncatalytic PTP-BL protein parts will therefore be required to determine the physiological impact of its various scaffolding and anchoring modules.

ACKNOWLEDGMENTS

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