insulin-resistant states such as obesity, PTP1B expression and activity are increased in muscle and adipose tissue of humans and rodents (16,17). Moreover, noncoding polymorphisms in the *PTPN1* gene have been found in different populations, displaying increased phosphatase expression in muscle associated with insulin resistance (18,19). In this regard, transgenic overexpression of PTP1B in muscle causes insulin resistance, showing impaired insulin signaling and decreased glucose uptake in this tissue (20). Furthermore, PTP1B overexpression in L6 muscle cells led to impaired insulin-stimulated IRS-1 phosphorylation and glycogen synthesis (21). By contrast, mice lacking PTP1B exhibit increased insulin sensitivity at 10–12 weeks of age (attributable to enhanced phosphorylation of insulin receptor in liver and skeletal muscle), resistance to weight gain on a high-fat diet, and an increased basal metabolic rate (22,23). In addition, treatment with PTP1B antisense oligonucleotide improves insulin sensitivity in *db/db* mice and increases insulin signaling in fat and liver in *ob/ob* mice (24,25). Furthermore, suppression of PTP1B in mouse embryo fibroblasts increases insulin signaling (26).

So far, no data are available on whether the lack of PTP1B might have an insulin-sensitizing effect under physiological and TNF-α–induced insulin-resistant conditions in skeletal muscle. To address this important issue, we have generated immortalized myocyte cell lines from wild-type and PTP1B-deficient neonatal mice. These cell lines are novel and unique tools to study the molecular mechanism of PTP1B-mediated insulin action in muscle because they express skeletal muscle markers, GLUT4, and the insulin signaling pathways known so far. PTP1B$^{-/-}$ myocytes displayed enhanced insulin stimulation on glucose uptake and on insulin receptor/IRS-1/phosphatidylinositol 3-kinase (PI 3-kinase)/AKT activation. Because treatment with TNF-α increases PTP1B expression and activity in wild-type myocytes, the lack of PTP1B confers protection against insulin resistance by TNF-α. Furthermore, this protective effect is also detected in vivo in the glucose and insulin tolerance tests performed in PTP1B-deficient adult mice treated with this cytokine.

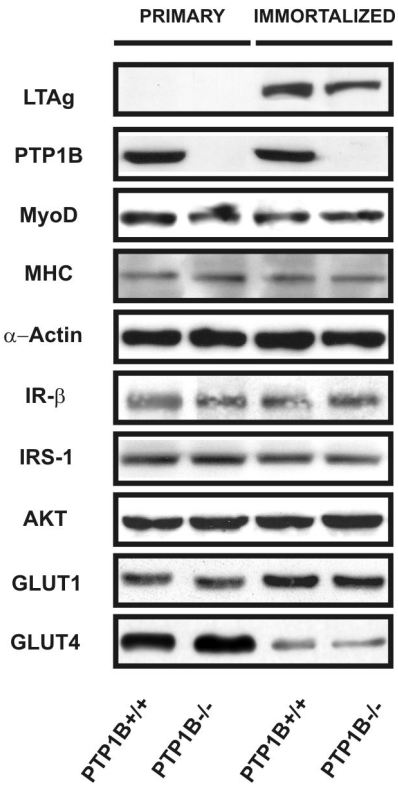

FIG. 1. Characterization of immortalized neonatal myocyte wild-type and PTP1B-deficient cell lines. Immortalized myocytes from wild-type (PTP1B$^{+/+}$) and PTP1B-deficient (PTP1B$^{-/-}$) newborn mice (3–5 days old) were generated as described in RESEARCH DESIGN AND METHODS. Cell lysates from immortalized myocytes cell lines and from primary myocytes were submitted to Western blot to analyze the expression of PTP1B, LTAg, Myo D, myosin heavy chain (MHC), α-actin, insulin receptor β-chain, IRS-1, AKT, GLUT4, and GLUT1. Representative immunoblots from three independent experiments are shown.

RESEARCH DESIGN AND METHODS

Animals and cell culture. Whole-body PTP1B-deficient and wild-type mice were obtained from Abbott Laboratories. In this model, the *Ptpn1* gene has been disrupted in coding exon 1, as described by Klaman et al. (23). All animal experimentation described in this study was conducted in accord with accepted standards of human animal care.

Pools of thigh muscles obtained from wild-type (PTP1B$^{+/+}$) and PTP1B$^{-/-}$ neonates (3–5 days old) were submitted to trypsinization and collagenase II dispersion and primary cultured in Dulbecco's minimal essential medium (DMEM) plus 10% horse serum (Invitrogen, Gaithersburg, MD), as previously described (13). Viral Bosc-23 packaging cells were transfected at 70% confluence with 3 μg/6-cm dish of the puromycin resistance retroviral vector pBabe encoding SV40 large T antigen (LTAg). Then, neonatal myocytes were infected at 60% confluence with polybrene (4 μg/ml)-supplemented virus for 48 h and maintained in culture medium for 72 h before selection with puromycin (0.5–1 μg/ml) for 1–2 weeks. Multiples dishes of infected cells were pooled to avoid potential clone-to-clone variations.

Immortalized cell lines were cultured in DMEM–10% fetal serum until reaching 90% confluence, shifted for 24 h to serum-free and low-glucose DMEM (1,000 mg/l) supplemented with 0.2% (wt/vol) BSA either in the absence or presence of 2 nmol/l TNF-α (Pharma Biotechnologie, Hannover, Germany), and further stimulated or not for 1–30 min with insulin (Sigma Chemical, St. Louis, MO) at different doses.

Glucose transport determination. Cells were stimulated for 30 min with insulin, and glucose uptake was measured during the last 10 min of culture by incorporation of 2-deoxy-D[1-^{3}H]-glucose (Amersham Bioscience, Little Chalfont, U.K.) as previously described (13). Then, monolayers were dissolved in 0.05 N NaOH, and aliquots were sampled for protein determination following Bradford protocol (Bio-Rad Laboratories) and for radioactivity measurements. Individual values were expressed as picomoles glucose per 10 min per milligram protein, and results were expressed as the percentage of stimulation over basal (control = 100).

Subcellular fractionation. Cells were submitted to subcellular fractionation for plasma membrane fraction isolation before protein quantification and Western blotting with GLUT4 and caveolin-1 antibodies as previously described (27).

Immunoprecipitations. Cells were lysed as previously described (13). After protein content determination, equal amounts of protein (600 μg to 1 mg) were immunoprecipitated at 4°C with different antibodies against IRSs and (P)-Tyr from Upstate Biotechnology (Lake Placid, NY) or insulin receptor β-chain (sc-711) from Santa Cruz (Palo Alto, CA). The immune complexes were collected on agarose beads and submitted to Western blot analysis. PI 3-kinase activity was measured in immunoprecipitates by in vitro phosphorylation of PI as previously described (27).

Western blotting. Analysis of protein expression was performed by Western blot as previously described (13), using the antibodies against GLUT1 and GLUT4 from Chemicon (Tamacula, CA); phosphorylated and total AKT and P70S6K from Cell Signaling (Beverly, MA); PTP1B, SH-PTP2, and protein phosphatase (PP)2A from Upstate Biotechnology; and MyoD (sc-760), P-Tyr (sc-508), insulin receptor β-chain (sc-09), caveolin-1 (sc-894), and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (sc-7974) from Santa Cruz. Immunoreactive bands were visualized using the enhanced chemiluminiscence (ECL-Plus) Western blotting protocol (Amersham).

Glucose and insulin tolerance tests. Male mice (10–12 weeks old) were treated for 48 h with TNF-α (0.1 μg/kg body wt, injected intraperitoneally) or vehicle (100 μl PBS plus 0.1% BSA). For glucose tolerance tests, mice fasted for 24 h were given an intraperitoneal injection of glucose (2 g/kg body wt). For insulin tolerance tests, fed animals were given an intraperitoneal injection of insulin (1 IU/kg body wt). Glucose concentration was determined in blood samples obtained from the tail vein at the indicated time points using an automatic analyzer (Accucheck; Roche).