Role of Protein Tyrosine Phosphatase Epsilon (PTP ϵ) in Leukotriene D₄-Induced CXCL8 Expression[§]

Fanny Lapointe, Sylvie Turcotte, Steeve Véronneau, Marek Rola-Pleszczynski, and Jana Stankova

Division of Immunology and Allergy, Department of Pediatrics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada

Received November 28, 2018; accepted March 6, 2019

ABSTRACT

Phosphorylation on tyrosine residues is recognized as an important mechanism for connecting extracellular stimuli to cellular events and defines a variety of physiologic responses downstream of G protein-coupled receptor (GPCR) activation. To date, few protein tyrosine phosphatases (PTPs) have been shown to associate with GPCRs, and little is known about their role in GPCR signaling. To discover potential cysteinyl-leukotriene receptor (CysLT₁R)-interacting proteins, we identified protein tyrosine phosphatase ϵ (PTP ϵ) in a yeast two-hybrid assay. Since both proteins are closely linked to asthma, we further investigated their association. Using a human embryonic kidney cell line 293 (HEK-293) cell line stably transfected with the receptor (HEK-LT1), as well as human primary monocytes, we found that PTP ϵ colocalized with CysLT₁R in both resting and leukotriene D₄ (LTD₄)-stimulated cells. Cotransfection of HEK-LT1 with PTP ϵ had

no effect on CysLT₁R expression or LTD₄-induced internalization, but it inhibited LTD₄-induced CXC chemokine 8 (CXCL8) promoter transactivation, protein expression, and secretion. Moreover, reduced phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2), but not of p38 or c-Jun-N-terminal kinase 1 or 2 mitogen-activated protein kinases (MAPKs), was observed upon LTD₄ stimulation of HEK-LT1 coexpressing cytosolic (cyt-) PTP ϵ , but not receptor (R) PTP ϵ . The increased interaction of cyt-PTP ϵ and ERK1/2 after LTD₄ stimulation was shown by coimmunoprecipitation. In addition, enhanced ERK1/2 phosphorylation and CXCL8 secretion were found in LTD₄-stimulated human monocytes transfected with PTP ϵ -specific siRNAs, adding support to a regulatory/inhibitory role of PTP ϵ in CysLT₁R signaling. Given that the prevalence of severe asthma is increasing, the identification of PTP ϵ as a new potential therapeutic target may be of interest.

Introduction

Numerous extracellular signals are transduced via phosphorylation-dephosphorylation cascades. Many kinases have been associated with G-protein-coupled receptor (GPCR) signaling, but relatively few protein tyrosine phosphatases (PTPs) have been associated with GPCRs, and little is known about their role in GPCR signaling; however, reports have established the involvement of both membrane-bound and cytosolic PTPs, with a few GPCRs having consequences in neurotransmitter signaling (Lopez et al., 1997; Florio et al., 1999; Tsai et al., 1999; Ferjoux et al., 2003; McCole et al., 2007;

Lee et al., 2013), mitogenic events (Rivard et al., 1995; Yu et al., 1997; Marrero et al., 1998; Duchene et al., 2002; Vatinel et al., 2006), cell migration, and tissue remodelling (Wang et al., 2009; Bakken et al., 2010). In addition, SHP-1, PTPRC, and PTPRJ play a role in inflammatory events through their effects on the chemokine receptor CXCR4 in lymphoid and myeloid cells (Kim et al., 1999; Vila-Coro et al., 1999; Ngai et al., 2009; Patrussi et al., 2014) and neutrophil fMLP receptor signaling (Zhu et al., 2011). Nevertheless, known PTPs acting downstream of GPCR signaling are relatively few, considering the high number of GPCR family members and the more than 100 distinct PTP genes (Alonso et al., 2004).

The cysteinyl-leukotrienes (cys-LTs), which comprise LTC₄, LTD₄, and LTE₄, have well characterized pathophysiological roles in the development of inflammatory diseases. For instance, their involvement in the pathology of asthma has been well established (Hay et al., 1995), along with the regulation of numerous inflammatory genes associated

This research was supported by the Canadian Institutes of Health Research [Grant MOP-142481] to J.S., and M.R.-P. F.L. is the recipient of a studentship from the Fonds de Recherche du Québec-Santé. The work was performed at the Centre de Recherche Clinique du Centre Hospitalier Universitaire de Sherbrooke, funded by the Fonds de la Recherche du Québec en Santé, of which M.R.-P. and J.S. are members.

<https://doi.org/10.1124/jpet.118.255422>.

[§] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: ANOVA, analysis of variance; AP-1, activator protein 1; BRET², bioluminescence resonance energy transfer; CXCL8, CXC motif chemokine ligand 8; Cys-LT, cysteinyl-leukotriene; CysLT₁R, cysteinyl-leukotriene receptor 1; ERK1/2, extracellular signal-regulated kinase 1/2; FBS, fetal bovine serum; GPCR, G-protein-coupled receptor; HEK-293, human embryonic kidney cells 293; HEK-LT1, human embryonic kidney cells stably expressing CysLT₁R; LTC₄, D₄, E₄, cysteinyl-leukotriene C4, D4, E4; LTD₄, leukotriene D4; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor-light-chain κ enhancer of activated B cells; pbFRET, photobleaching fluorescence resonance energy transfer; PCR, polymerase chain reaction; PTP, protein tyrosine phosphatase; PTP ϵ , protein tyrosine phosphatase ϵ ; RPTP, receptor-type phosphatase; TNF α , tumor necrosis factor α ; TRITC, tetramethylrhodamine.

with its development and progression (Rola-Pleszczynski and Stankova, 2007). For example, we have shown that LTD $_4$, via its high-affinity receptor, CysLT $_1$ R (Lynch et al., 1999), transcriptionally induces the production of the chemokine CXCL8 in the human monocytic cell line THP-1 and human dendritic cells (Thompson et al., 2006). CXCL8, as a potent chemoattractant and activator of neutrophils, is involved in severe forms of asthma and is considered a marker of severity for the disease (Silvestri et al., 2006; Turkeli et al., 2015).

In our studies to further define the signaling mechanisms of CysLT $_1$ R, we identified PTP ϵ , a classic receptor-type phosphatase (Alonso et al., 2004), as a novel CysLT $_1$ R-interacting protein in a yeast two-hybrid assay. PTP ϵ is represented by five different isoforms: the receptor-type (RPTP ϵ) and four nontransmembrane, cytoplasmic (cyt)-PTP ϵ isoforms (cyt-PTP ϵ , p67 (Gil-Henn et al., 2000), p65 (Gil-Henn et al., 2001), and cyt-PTP ϵ D1 (Wabakken et al., 2002b)). RPTP ϵ and cyt-PTP ϵ are the most abundantly expressed isoforms, and both are the products of a single gene via the use of alternative promoters (Tanuma et al., 1999).

The cell- or stimulus-specific role of PTP ϵ in inflammatory responses is still unclear; however, macrophages from PTP $\epsilon^{-/-}$ mice show a reduction in the generation of reactive oxygen species and tumor necrosis factor α (TNF α) secretion after stimulation with LPS (Sully et al., 2001), whereas mast cells exhibit an increase in degranulation and TNF α secretion after Fc ϵ R1 receptor activation (Akimoto et al., 2009). Both PTP ϵ isoforms were also studied for their inhibition of extracellular signal regulated kinase 1 or 2 (ERK1/2) and Shc kinases and their association with Grb2 (Toledano-Katchalski and Elson, 1999; Wabakken et al., 2002a; Toledano-Katchalski et al., 2003; Kraut-Cohen et al., 2008; Levy-Apter et al., 2014).

An estimated 300 million people suffer from asthma worldwide, and this affliction is expected to increase in the next decades (Masoli et al., 2004). Roughly, 20% of these patients have severe asthma, of which 20% are inadequately controlled despite treatment guidelines (Rabe et al., 2004). Thus, the identification of new therapeutic targets is necessary. Our findings suggest that PTP ϵ may be an important regulator of inflammatory processes initiated by cys-LTs, and a better understanding of the interaction between CysLT $_1$ R and PTP ϵ may further define their involvement in asthma. Moreover, a polymorphism in the *PTPRE* gene has been associated with allergic asthma (Tremblay et al., 2008), potentially making its interaction with CysLT $_1$ R even more significant.

The objective of this work was to determine, by using multiple techniques, whether PTP ϵ colocalizes with CysLT $_1$ R. In addition, we studied the mechanism of PTP ϵ -dependent regulation of CXCL8 expression after LTD $_4$ stimulation.

Materials and Methods

Antibodies and Reagents. Antibodies against PTP ϵ were purchased from GenWay Biotech, Inc. (20-614-460700; San Diego, CA) and Abcam PLC (ab123345; Toronto, ON). Cayman Chemical Company (120500; Ann Arbor, MI) provided the anti-CysLT $_1$ R antibodies; anti-Myc 9E10 antibodies were from the American Type Culture Collection (ATCC) (Manassas, VA). Mouse anti-human CD14 (347490) were from BD Biosciences (Mississauga, ON). Fluorescent secondary antibodies, goat anti-rabbit-AlexaFluor 488 (111-547-003), and goat

anti-mouse-tetramethylrhodamine (TRITC) (115-096-146) were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Goat anti-mouse AlexaFluor 488 (A-11017) was from Thermo Scientific (Lafayette, CO). DAPI was purchased from Molecular Probes (Burlington, ON). Specific antibodies against FLAG (F3165) and vinculin (V4505) were purchased from Sigma-Aldrich (Oakville, ON); anti-p-ERK1/2 (sc-7383) and anti-ERK1 (sc-93) antibodies were from Santa Cruz Biotechnologies, Inc. (Santa Cruz, CA). Secondary antibodies conjugated to horseradish peroxidase (HRP) used in Western blot detection were from Cell Signaling Technology (7074, 7076) (Danvers, MA).

LTD $_4$ and PD98059 were purchased from Cayman Chemical Company. Coelenterazine 400a was acquired from Biotium, Inc. (Hayward, CA). Leupeptin, aprotinin, phenylmethanesulfonyl fluoride, pepstatin A and the Phosphatase Inhibitor Cocktail II (PICII) were from Sigma-Aldrich. Complete Mini EDTA-free protease inhibitor tablets were from Roche Diagnostics (Laval, QC, Canada). Finally, the transfection reagent used to transiently transfect HEK-293 and HEK-LT1 cells was TransIT-LT1 (Mirus Bio LLC, Madison, WI), whereas human monocytes were nucleofected with the Human Monocyte Nucleofector Kit (Cedarlane, Burlington, ON). Immunoprecipitations were performed with the Dynabeads Protein A Immunoprecipitation kit (Thermo Scientific).

Plasmids. Plasmids used in this work were constructed in our laboratory; cyt-PTP ϵ and RPTP ϵ , stabilized by a 5' β -globin intron and under the control of a cytomegalovirus promoter, were subcloned into pcDNA3 vector. A FLAG epitope was also added to this construction with *substrate trapping* mutations (RPTP ϵ ; D303A, cyt-PTP ϵ ; D245A). Cyt-PTP ϵ and RPTP ϵ were also C-terminus-tagged with Venus-C (C-terminal half of Venus fluorescent protein; residues 156–239). Both PTP ϵ isoforms were also individually subcloned into pGFP 2 -N3(h) vector to yield C-terminus-tagged Cyt-PTP ϵ -GFP 2 and C-terminus-tagged RPTP ϵ -GFP 2 .

Myc_CysLT $_1$ R and Venus-N_CysLT $_1$ R (N-terminal half of Venus fluorescent protein; residues 1–155) were subcloned into the pcDNA3 vector. CysLT $_1$ R was also subcloned in the pRluc-N3(h) vector. The resulting N-terminus-tagged myc_CysLT $_1$ R and Venus-N_CysLT $_1$ R, as well as CysLT $_1$ R_Rluc constructs, targeted the plasma membrane correctly. The human CXCL8 promoter-luciferase construct was provided by Dr. Allan R. Brasier, University of Texas Medical Branch (Vlahopoulos et al., 1999).

Finally, siRNAs used in this study were from Ambion (Burlington, ON).

Cells. HEK-293 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (Gibco, Burlington, ON) supplemented with 5% fetal bovine serum (FBS) (PAA, Piscataway, NJ). HEK-LT1:HEK-293 cells stably expressing CysLT $_1$ R have been described in Thompson et al. (2006). Experiments were performed 48 hours post-transfection in HEK-293 and HEK-LT1 cells.

Human primary monocytes were isolated from peripheral blood mononuclear leukocytes obtained from healthy donors after informed written consent, in accordance with an internal review board-approved protocol (no. 2016-1167-CysLT), according to the technique described by Böyum (1968) and as previously described (Thivierge et al., 2001). peripheral blood mononuclear leukocytes were first enriched by sedimentation using Dextran T500 (Pharmacosmos, Holbaek, Denmark), followed by density-gradient centrifugation on Ficoll-Paque Plus (GE Healthcare Life Sciences, Mississauga, ON). Mononuclear cells were collected at the interface of the Ficoll, and monocytes were isolated by adherence using Petri dishes previously coated with defibrinized autologous serum. Monocytes were resuspended in RPMI 1640 medium (Gibco) supplemented with 5% FBS. Experiments were performed after overnight incubation of human monocytes after their isolation or 48 hours postnucleofection. Cells were grown under normal conditions in a humidified atmosphere with 5% CO $_2$ at 37°C.

Laser Scanning Confocal Microscopy. Cell preparation for laser scanning confocal microscopy was done with minor modification of a previously published protocol (Hamel-Cote et al., 2017). Briefly,

HEK-293 cells, transiently cotransfected with myc_CysLT₁R and PTP_ε-GFP², were grown on pretreated coverslips (0.1 mg/ml poly-L-lysine). Forty-eight hours posttransfection, adherent cells were fixed with 2% paraformaldehyde and permeabilized with 0.1% saponin. A 2% BSA solution was used to block nonspecific sites. Cells were then incubated with primary antibody for 30 minutes (anti-Myc 9E10), followed by incubation with the appropriate secondary antibody (anti-mouse-TRITC (115-096-146), 1:1000) for an additional 30 minutes. Human monocytes were incubated with antibodies against PTP_ε (20-614-460700, 1:60) and CysLT₁R (120500, 1:25) and revealed with the appropriate secondary antibodies (anti-rabbit-AlexaFluor 488 (111-547-003), 1:750, anti-mouse-TRITC (115-096-146), 1:750). DAPI staining was used to visualize the nucleus. Finally, cells were visualized and analyzed using an Olympus FluoView FV1000 confocal microscope (Center Valley, PA). Captured images were further analyzed using Image-Pro Plus 6.0 from MediaCybernetics (Bethesda, MD).

Bimolecular Fluorescence Complementation Assay. HEK-293 cells transiently coexpressing Venus-N_CysLT₁R and PTP_ε-Venus-C were grown on pretreated coverslips (0.1 mg/ml poly-L-lysine). Forty-eight hours post-transfection, adherent cells were treated as previously described and analyzed using confocal microscopy.

Bioluminescence Resonance Energy Transfer Curves. Bioluminescence resonance energy transfer (BRET²) saturation assays were done as previously described (Poisson et al., 2009). HEK-293 cells were grown in six-well plates and cotransfected with a constant amount of CysLT₁R_Rluc and increasing amounts of PTP_ε-GFP². For competition curves, HEK-293 cells were cotransfected with a constant amount of CysLT₁R_Rluc and PTP_ε-GFP² and increasing amounts of nontagged PTP_ε. BRET² signal was measured 48 hours post-transfection; cells were harvested, resuspended in BRET² buffer (phosphate-buffered saline, 1 g/l glucose), transferred into 96-wells plates (OptiPlate; PerkinElmer, Waltham, MA), and incubated in the presence or absence of ligands. For dose-response curves, the plates were scanned 15 minutes after agonist addition. The substrate Coelenterazine 400a was added at a final concentration of 5 μM, and readings at 410/80 nm (bioluminescence given off by Rluc) and 515/30 nm (resonance energy transfer from Rluc to GFP²) were measured simultaneously. Bioluminescence readings were corrected for those obtained from cells transfected with empty vectors. The BRET² ratios were calculated as the ratio of light emitted by GFP² (515/30 nm) over the light emitted by the Rluc (410/80 nm). Ratios reported were then corrected by subtracting the ratios obtained when Rluc was expressed alone. Analysis was performed on a PerkinElmer Fusion spectrometer.

Photobleaching Fluorescence Resonance Energy Transfer. Human monocytes were treated and stained as previously described. Specifically, the fluorescent dyes AlexaFluor 488 (excitation: 495 nm, emission: 519 nm) (anti-rabbit-AlexaFluor 488 (111-547-003), 1:750) and TRITC (excitation: 544 nm, emission: 572 nm) (anti-mouse-TRITC (115-096-146), 1:750) were used as the donor and acceptor fluorescent proteins to tag CysLT₁R and PTP_ε (or CD14), respectively. For the photobleaching fluorescence resonance energy transfer (pbFRET) experiment, cells were first visualized using confocal microscopy and then photobleached at 544 nm. Bleached cells were then visualized at the donor excitation wavelength (495 nm).

RNA Isolation and Reverse Transcription-Polymerase Chain Reaction. Total RNA was purified using Trizol Reagent (Thermo Scientific) according to the manufacturer's instructions using the classic phenol/chloroform technique or with the RNeasy Micro Kit (Qiagen Inc., Toronto, ON) when using nucleofected monocytes. To exclude genomic DNA contamination, RNA was digested with gDNA Wipeout, provided in the QuantiTect Reverse Transcription Kit (Qiagen Inc.). First-strand cDNA synthesis was performed on 1 μg of RNA using random primers supplied in the aforementioned kit.

Real-Time Quantitative Polymerase Chain Reaction. Real-time quantitative polymerase chain reaction (PCR) was performed using the Rotor Gene RG-3000 from Corbett Research (San Francisco,

CA). We mixed 25 ng of cDNA with 0.5-μM primers (IDT, Coralville, IA), 1.25 U of TAQ DNA polymerase (Feldan, Quebec, QC), 0.2 mM dNTPs (Feldan), 1 mM MgCl₂ (Thermo Scientific), and 1:32,500 SYBR Green (Molecular Probes), final dilution in supplied TAQ DNA polymerase buffer, for a final volume of 52 μl. PCR was started with a 5-minutes hold step at 95°C, followed by 35 times repeat of the cycling step: 30 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C (acquiring on Fam/syb), and ended by a melt step rising from 72°C to 95°C. Data analysis was performed according to the 2^{ΔΔCT} method as previously described (Dussault and Pouliot, 2006). The primer sequences were the following:

RPL13A forward 3'-GTGCGTCTGAAGCCTACAAG-5',
RPL13A reverse 3'-TCTTCTCCAGTTCTTCTCG-5',
GAPDH forward 3'-TCAACGGATTGGTCGTATTGG-5',
GAPDH reverse 3'-GATGGGATTTCATTGATGACA-5',
cyt-PTP_ε forward 3'-CTTTTCCCGGCTCACCTGGTTC-5',
cyt-PTP_ε reverse 3'-GGATGGGAAAATACTTCTTGG-5',
RPTP_ε forward 3'-GCCTACTTCTTCAGGTTCTCAGG-5',
RPTP_ε reverse 3'-GGATGGGAAAATACTTCTTGG-5',
CXCL8 forward 3'-TTCTGCAGCTCTGTGTGAAG-5',
CXCL8 reverse 3'-AAACTTCTCCACAACCCTCTG-5'.

Western Blots. Western blots were done with minor modification of a previously published protocol (Boudaud et al., 2018). Briefly, human monocytes and HEK-LT1 cells were lysed using lysis buffer containing 1% NP-40 [20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 1% NP-40, 10% glycerol] and containing the inhibitors 1 mM phenylmethanesulfonyl fluoride, 2 μg/ml aprotinin, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, PICII 1X, and a Complete Mini EDTA free protease inhibitor tablet, for 30 minutes on ice. Total protein concentrations were quantified using Pierce Coomassie Plus assay kit (Thermo Scientific), and 30 μg was separated on a 10% SDS-PAGE and transferred onto a 0.45 μM nitrocellulose membrane (GE Healthcare Life Sciences). The membrane was incubated with the primary antibodies overnight at 4°C [PTP_ε (ab123345), 1:1000] [p-ERK1/2 (sc-7383), 1:1000] [vinculin (V4505), 1:2500] in a TBS + 0.05% Tween-20 + 5% BSA for phosphorylated proteins or a TBS + 0.05% Tween-20 + 5% free-fat milk solution for nonphosphorylated proteins. Proteins were detected subsequent to a 30-minute incubation with secondary antibodies labeled with HRP (anti-mouse (7076), anti-rabbit (7074), 1:2500) using an ECL detection system (GE Healthcare Life Sciences) on a ChemoDoc MP Imaging System (Bio-Rad, Mississauga, ON, Canada). Signal intensity was quantified by densitometry using Image Laboratory. After phosphorylated protein detection, membranes were stained for total proteins subsequent to a 20-minute stripping protocol [200 mM glycine, 3.5 mM SDS, 1% Tween-20 (pH 2.2)] [ERK1/2 (sc-93), 1:1000].

Coimmunoprecipitation. HEK-LT1 cells were transiently transfected with substrate-trapping mutants of FLAG-tagged PTP_ε and then stimulated as indicated. Cells total lysates (200 μg) were incubated for 1 hour 30 minutes at 4°C, with constant rotation, with Dynabeads Protein A previously coated with anti-FLAG antibody (8 μg), according to the manufacturer's instructions. Coimmunoprecipitates were eluted in 20 μl 50 mM glycine [pH 2.8] and 15 μl were separated on a 10% SDS-PAGE. Subsequent analysis of the coimmunoprecipitates was performed as described in the preceding section.

Luciferase Assays. CXCL8 promoter transactivation assays were performed as described by Thompson et al. (2006). HEK-293 or HEK-LT1 cells were grown in 12- or 24-wells plates and transiently cotransfected with CysLT₁R, pcDNA3, or PTP_ε in addition to the pCXCL8_Rluc constructs. The day after the transfection, cells were serum-starved overnight before a 6-hour stimulation with EtOH or LTD₄, at indicated concentrations and then lysed with 25 mM Gly-Gly, 7.6 mM MgSO₄, 4 mM EGTA, 1% Triton X-100, and 2 mM dithiothreitol. Cell lysates were assayed for luciferase activity with 1.375 mM ATP (Sigma-Aldrich) and 0.187 mM D-Luciferin Na-Salt (Molecular Probes) using a Sirius Luminometer (Titertek-Berthold, Pforzheim, Germany).

siRNA Nucleofection. Human monocytes were nucleofected according to the manufacturer's specifications (Cedarlane). Briefly, 6×10^6 cells were washed with phosphate-buffered saline + 0.5% BSA and gently suspended in 100 μ l-supplemented nucleofection solution, and 50 pmol of siRNAs. Monocytes were nucleofected using a Nucleofector II device (program Y-001) and immediately resuspended in 6 ml of RPMI + 10% FBS. Experiments were performed 48 hours after siRNA nucleofection.

Enzyme-Linked Immunosorbent Assay. HEK-LT1 cells were grown in 12-well plates and transiently transfected with pcDNA3 or PTP ϵ . The day after transfection, cells were serum-starved overnight before a 20-hour stimulation with EtOH or LTD $_4$ at indicated concentrations. CXCL8 was measured in cell-free supernatants with the human CXCL8 enzyme-linked immunosorbent assay MAX Deluxe Set (BioLegend, San Diego, CA) according to the manufacturer's recommendations.

Statistical Analyses. Student's *t* test and two-way analysis of variance (ANOVA) analyses were performed when appropriate using Prism 7.0 software (GraphPad Software, San Diego, CA). *P* < 0.05 was considered statistically significant.

Results

Human Monocytes Express Multiple Isoforms of PTP ϵ . In most murine cell types examined, the expression of cyt-PTP ϵ and RPTP ϵ is mutually exclusive. Given the multitude of potential isoforms and that PTP ϵ expression has not been studied in detail in human cells, we examined the expression of PTP ϵ isoforms in freshly isolated human monocytes. As shown in Fig. 1A, the molecular weights of the expressed proteins suggest the expression of RPTP ϵ , cyt-PTP ϵ , and p67^{PTP ϵ} in these cells. Also, an isoform, which could potentially be cyt-PTP ϵ PD1, the result of alternative splicing (Wabakken et al., 2002b), is expressed in 2 of the samples from healthy donors.

Unlike human monocytes, HEK-LT1 cells do not express PTP ϵ ; thus, these cells were transiently transfected with expression vectors for cyt-PTP ϵ or RPTP ϵ cDNA. The resulting transient expression of the phosphatase in this model allowed us to compare the multiple isoforms with those expressed in human monocytes. As shown in Fig. 1B, the transfection of HEK-LT1 cells with the expression vector of cyt-PTP ϵ resulted in the expression of cyt-PTP ϵ and p67^{PTP ϵ} . Transfection of HEK-LT1 cells with the expression vector for RPTP ϵ resulted in the expression of multiple PTP ϵ isoforms with molecular weights ranging from 63 to 91 kDa. Higher-weight isoforms could represent the multiple glycosylated forms of RPTP ϵ (Supplemental Fig. 1). HEK-LT1 cells transiently expressing RPTP ϵ were also expressing p67^{PTP ϵ} and p65^{PTP ϵ} . p67^{PTP ϵ} is produced by

an alternate initiation codon corresponding to Met28 of cyt-PTP ϵ and Met86 of RPTP ϵ (Gil-Henn et al., 2000); it can thus be expressed after the transient transfection of vectors for both cyt-PTP ϵ and RPTP ϵ . Lastly, the p65^{PTP ϵ} isoform can result from calpain proteolytic processing of larger PTP ϵ molecules (Gil-Henn et al., 2001). Of note, lysis of HEK-LT1 cells was performed in the presence of multiple protease inhibitors, including calpain inhibitors, indicating that p65^{PTP ϵ} was present before cell lysis. Although multiple PTP ϵ isoforms were expressed after transient transfection of HEK-LT1 cells with cyt-PTP ϵ or RPTP ϵ , the latter were most abundant. It should also be highlighted that cyt-PTP ϵ PD1 was only detected in human monocytes, whereas p65^{PTP ϵ} only in HEK-LT1 transiently transfected with the RPTP ϵ expression vector.

PTP ϵ expression was similar both in HEK-293 and in HEK-LT1 cells used for the following experiments (Supplemental Fig. 2). Likewise, antibodies used in Western blotting revealed the same PTP ϵ pattern of expression as the GFP² tag used in confocal imaging (Supplemental Fig. 2).

PTP ϵ and CysLT $_1$ R Share a Similar Localization. After the yeast two-hybrid assay results, PTP ϵ and CysLT $_1$ R interaction in the cell was supported by colocalization studies using confocal microscopy imaging. HEK-293 cells, transiently coexpressing CysLT $_1$ R and PTP ϵ , were stained with appropriate antibodies (Fig. 2A). Analysis of the confocal microscopy images showed that cyt-PTP ϵ distribution was throughout the cell, whereas RPTP ϵ was found mainly at the cell membrane, in agreement with previously published data of PTP ϵ cellular distribution (Elson and Leder, 1995; Andersen et al., 2001; Sines et al., 2007). CysLT $_1$ R and PTP ϵ colocalized at the plasma membrane.

Colocalization of the two proteins was also found in human primary monocytes (Fig. 2B). Although in these cells we could not distinguish between cyt-PTP ϵ and RPTP ϵ , strong colocalization was observed around the nucleus.

To further illustrate this interaction, bimolecular fluorescence complementation was used with HEK-293 cells transiently coexpressing Venus-N_CysLT $_1$ R and PTP ϵ -Venus-C. Using this assay, fluorescence can be seen only if Venus-N and Venus-C are close enough to each other to reconstruct the fluorescent protein. As presented in Fig. 2C, Venus fluorescence was evident at the level of both the membrane and the cytoplasm of cells cotransfected with cyt-PTP ϵ and RPTP ϵ . Hence, using this irreversible protein reconstruction technique, we could illustrate the proximity of CysLT $_1$ R and PTP ϵ in HEK-293 cells.

Fig. 1. Expression of multiple PTP ϵ isoforms in human monocytes and transiently transfected HEK-LT1 cells. (A) Freshly isolated human monocytes and (B) HEK-LT1 cells, transiently transfected with cyt-PTP ϵ or RPTP ϵ for 48 hours, were lysed, and lysates were used for Western blots with anti-PTP ϵ antibodies to detect PTP ϵ isoforms according to their molecular weights. Representative Western blots are shown.

Fig. 2. Colocalization of CysLT₁R with cyt-PTP ϵ and RPTP ϵ in HEK-293 cells and human monocytes. (A) HEK-293 cells were transiently cotransfected with cyt-PTP ϵ -GFP² or RPTP ϵ -GFP². Forty-eight hours post-transfection, cells were fixed, permeabilized, and stained with antibodies against c-Myc (CysLT₁R – red); PTP ϵ (cyt-PTP ϵ and RPTP ϵ) was detected with its GFP² tag (green). (B) Human monocytes were fixed, permeabilized, and stained with antibodies against CysLT₁R (red) and PTP ϵ (green). (C) HEK-293 cells were transiently cotransfected with Venus-N_CysLT₁R and cyt-PTP ϵ -Venus-C or RPTP ϵ -Venus-C. Forty-eight hours post-transfection, cells were fixed and permeabilized. Fluorescence reconstitution could be seen only if the two Venus constructs (N-terminal and C-terminal) were adjacent within the cells. Fluorescence was observed by laser scanning confocal microscopy, and DAPI staining was used to visualize the nucleus. The masks (A and B) illustrate the pixels that colocalize. Scale bar, 15 μ M.

To address more completely the colocalization of PTP ϵ and CysLT₁R in human monocytes, we used photobleaching fluorescence resonance energy transfer (pbFRET). Cells were treated as previously described for laser scanning confocal microscopy imaging, and the colocalization partners were identified with their appropriate antibodies and stained according to the overlapping fluorescence spectrum of the dyes (Ishikawa-Ankerhold et al., 2012). Thereby, staining the monocytes with AlexaFluor 488 (PTP ϵ : donor) and TRITC (CysLT₁R: acceptor) allowed us to visualize the fluorescence energy transfer from PTP ϵ to CysLT₁R (Fig. 3, A and A'). Hence, when TRITC (CysLT₁R), the acceptor, was photobleached, a reduction in its fluorescence

was seen, and donor (AlexaFluor 488's PTP ϵ) fluorescence was increased ("dequenched"), as shown in Fig. 3, A and A' in the FRET efficiency column. No FRET could be seen when staining the cluster of differentiation 14 (CD14), a cell surface receptor and differentiation marker of monocytes, instead of PTP ϵ (Fig. 3B).

Cyt-PTP ϵ Has a Strong Affinity for CysLT₁R. Bioluminescence resonance energy transfer (BRET²) experiments allowed us to study, simultaneously, the proximity and the affinity between PTP ϵ and CysLT₁R in real time. Using HEK-293 cells transiently cotransfected with CysLT₁R_Rluc and PTP ϵ -GFP², we used saturation and competition assays to ensure specificity of the interactions. Figure 4A shows the

Fig. 3. FRET with acceptor photobleaching indicates that PTP ϵ and CysLT $_1$ R are near human monocytes. Human primary monocytes were fixed, permeabilized, and stained with antibodies against CysLT $_1$ R and (A and A') PTP ϵ or (B) CD14, as a negative control. The secondary antibodies, AlexaFluor 488 (PTP ϵ , CD14) and TRITC (CysLT $_1$ R), were chosen according to their fluorescence spectrum to be the photon donor and acceptor, respectively. A close interaction between the proteins is suggested as AlexaFluor 488 (PTP ϵ) shows an increased level of fluorescence after a section of cell (marked by rectangles) is irradiated to photobleach the acceptor TRITC (CysLT $_1$ R, middle panels). The subsequent increase in donor fluorescence is defined by a mask in white (right panels). No FRET is observed between CD14 and CysLT $_1$ R. Fluorescence was observed by laser scanning confocal microscopy. Scale bar, 5 μ M.

saturation of both cyt-PTP ϵ - and RPTP ϵ -CysLT $_1$ R curves, indicating the specificity of the interactions. Furthermore, one-site specific binding BRET 2 curves illustrate the difference between cyt-PTP ϵ and RPTP ϵ binding with CysLT $_1$ R. Dimerization of CysLT $_1$ R with cyt-PTP ϵ gives a BRET $_{50}^2$ of 1.345 ± 0.6255 , and dimerization of CysLT $_1$ R with RPTP ϵ , a BRET $_{50}^2$ of 7.666 ± 3.478 . Hence, even if HEK-293 cells tend to express more RPTP ϵ than cyt-PTP ϵ (as shown by the form of the curve on the abscissa), cyt-PTP ϵ presents a stronger colocalization with CysLT $_1$ R. BRET 2 competition curves gave similar results. To that end, HEK-293 cells were transiently cotransfected with a constant amount of CysLT $_1$ R_Rluc, as done previously, in addition to a constant amount of PTP ϵ _GFP 2 and increasing amounts of nontagged PTP ϵ . Stronger colocalization of cyt-PTP ϵ with CysLT $_1$ R was demonstrated when compared with RPTP ϵ , as the one-site fit logIC $_{50}$ curves resulted in IC $_{50}$ of 4.931 ± 0.5253 and 3.559 ± 0.5130 for cyt-PTP ϵ and RPTP ϵ , respectively (Fig. 4B). CysLT $_1$ R and PTP ϵ interaction showed no significant change upon LTD $_4$ stimulation (Fig. 4C). Of note, neither cyt-PTP ϵ nor RPTP ϵ colocalized with the cluster of differentiation 8 (CD8), another membrane receptor-type protein (Supplemental Fig. 3).

Taken together, these results demonstrate that PTP ϵ associates with CysLT $_1$ R, both in transfected cells and in human monocytes. Moreover, BRET 2 curves using transiently transfected HEK-293 cells indicated a higher affinity of cyt-PTP ϵ for CysLT $_1$ R.

PTP ϵ Inhibits LTD $_4$ -Induced CXCL8 Expression.

Since GPCR and PTP interactions have not been widely studied, the identification of a phosphatase as an interacting partner of CysLT $_1$ R could shed light on new actors in the regulation of GPCR function, as well as their surface expression. In fact, GPCR surface expression is known to be tightly regulated, partly by phosphorylation (Tobin, 2008). CysLT $_1$ R surface expression was quantified after transient expression of PTP ϵ in HEK-LT1 cells. Although HEK-LT1 cells expressed both PTP ϵ mRNA isoforms in a concentration-dependent manner (Supplemental Fig. 4, A and B), they tended to express more RPTP ϵ than cyt-PTP ϵ , as demonstrated by Western blot densitometry quantification (Supplemental Fig. 4, C and D). This differential expression was supported by the BRET 2

saturation curves (Fig. 4A). Nevertheless, flow cytometry experiments revealed that CysLT $_1$ R surface expression was not affected by the expression of either isoform (Supplemental Fig. 4, E and F). Additionally, transient PTP ϵ expression had no effect on CysLT $_1$ R internalization after LTD $_4$ stimulation of HEK-LT1 cells (Supplemental Fig. 4, G and H). Thus, these results indicate that, on a basal level, PTP ϵ could be in proximity to CysLT $_1$ R without interfering with its surface expression.

Next, we addressed the potential role of the phosphatase in LTD $_4$ -induced chemokine expression. We have previously shown that activation of CysLT $_1$ R by LTD $_4$ induces CXCL8 production (Thompson et al., 2006). Thus, we investigated whether PTP ϵ could be involved in LTD $_4$ -induced CysLT $_1$ R signaling pathway for CXCL8 production. As shown in Fig. 5A, LTD $_4$ -induced CXCL8 promoter transactivation diminished with increasing PTP ϵ concentrations. Responses to all concentrations of LTD $_4$ were also significantly decreased compared with control (Fig. 5B). Correspondingly, CXCL8 mRNA expression and protein secretion were inhibited in HEK-LT1 cells transiently transfected with PTP ϵ (Fig. 5, C and D). The isoforms displayed different inhibition levels; however, they were not expressed at the same levels in the cell model used for these experiments, which may account for this difference. No synergy or additive inhibition resulted from a combination of both isoforms (Supplemental Fig. 5).

Subsequent to CXCL8 experiments in HEK-LT1 cells, we investigated the potential role of PTP ϵ in human primary monocytes. Since we had previously shown LTD $_4$ -induced secretion of CXCL8 in the monocytic cell line THP-1 and in human dendritic cells (Thompson et al., 2006), we were interested in CXCL8 secretion in human monocytes. These cells, obtained from healthy donors and stimulated with graded-concentrations of LTD $_4$, responded with the characteristic bell-shaped curve typical of GPCR stimulation (data not shown).

Nucleofection of monocytes with siRNAs designed against both PTP ϵ isoforms led to a reduction of 22% and 21% in cyt-PTP ϵ and RPTP ϵ mRNA expression, respectively (Supplemental Fig. 6B). Similarly, Western blot experiments showed a decreased cyt-PTP ϵ protein expression by 23% and RPTP ϵ by 22% in siRNA-nucleofected monocytes (Supplemental

Fig. 4. Cyt-PTPε has a stronger affinity for CysLT₁R than RPTPε in transiently cotransfected HEK-293 cells. HEK-293 cells were cotransfected with a constant amount of CysLT₁R_Rluc and (A) increasing amounts of cyt-PTPε_GFP² (gray line) or RPTPε_GFP² (black line) for saturation curves and (B) constant amount of PTPε_GFP² and increasing amount of nontagged PTPε for competition curves. Data shown are the mean of multiple experiments as a function of GFP²/Rluc expression. Data were plotted using GraphPad Prism and the B_{max} and K_d determinations were taken as the BRET_{max}² and the BRET₅₀², respectively ($n = 6$). IC₅₀ is shown in BRET² competition assay ($n = 6$). (C) The BRET² signals of LTD₄-stimulated conditions [Log, (LTD₄), M] (15 minutes) were plotted over the nonstimulated condition (100%) ($n = 4$). Data are expressed as mean (A) or mean ± S.E.M. (B and C).

Fig. 6C). These slight decreases of expression were the best results we obtained, no matter how we optimized the experiments (different siRNAs, concentrations, timing, etc). We hypothesized that PTPε may have a long half-life in human monocytes, making it difficult to downregulate this protein within these cells; however, monocytes treated with

actinomycin D (Supplemental Fig. 6D) and cycloheximide (Supplemental Fig. 6E) indicated a mRNA half-life less than 4.5 hours and a protein half-life less than 2.5 hours, without significant differences between isoforms, although cyt-PTPε tended to have a slightly shorter half-life than RPTPε. Therefore, the phosphatase may have a high production rate,

Fig. 5. Concentration-dependent decrease of LTD₄-induced CXCL8 expression by PTPε. (A) HEK-293 cells were transiently cotransfected with CysLT₁R, pCXCL8_Rluc and increasing amounts of pcDNA3, cyt-PTPε (gray line) or RPTPε (black line). Cells were stimulated with EtOH (vehicle) or LTD₄ (10 nM) for 6 hours, and luciferase activity assays were performed ($n = 5$). (B and C) HEK-LT1 cells, transiently transfected with 100 ng of pcDNA3 (dotted line), cyt-PTPε (gray line), or RPTPε (black line), were stimulated with EtOH or LTD₄, as indicated, for 6 hours and (B) luciferase assays or (C) RT-qPCRs were performed ($n = 4$). CXCL8 mRNA expression was quantified as $2^{\Delta\Delta CT}$ over RPL13A mRNA expression ($n = 7$). (D) For measurement of CXCL8 protein secretion, HEK-LT1 cells transiently transfected with 100 ng PTPε were stimulated for 20 hours with EtOH or LTD₄, as indicated, and enzyme-linked immunosorbent assay was used to quantify CXCL8 protein production in cell-free supernatants ($n = 5$). (E) 48 hours after nucleofection with the corresponding siRNAs—siCTRL (dotted line) or siPTPε (black line)—human primary monocytes were stimulated with EtOH or LTD₄, as indicated, for 6 hours. CXCL8 mRNA expression was quantified as previously described ($n = 7-9$). Data are expressed as mean ± S.E.M. * $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$; **** $P < 0.0001$, by (A) two-way ANOVA with Sidak's multiple comparisons post-test and (B–D) two-way ANOVA with Dunnett's multiple comparisons post-test to compare cyt-PTPε or RPTPε with control (pcDNA3).

explaining our difficulties in reducing its expression. Still, the decreased expressions with our siRNAs were consistent, so they were used in subsequent experiments. Monocytes nucleofected with control and PTP ϵ -specific siRNAs were stimulated with LTD $_4$. Using real-time quantitative PCR analysis, we observed a trend to increased LTD $_4$ -induced CXCL8 mRNA expression in PTP ϵ -specific siRNA compared with control siRNA-nucleofected cells (Fig. 5E). No significant impact on the basal expression of the chemokine or CysLT $_1$ R expression was observed in these paired siRNA-nucleofected monocytes, as expressed by mean fluorescence intensity through flow cytometry analysis (Supplemental Fig. 6, F and G).

PTP ϵ Inhibits ERK1/2 MAPK Activation. To study the mechanism of PTP ϵ -mediated inhibition of CXCL8 expression, we examined LTD $_4$ -induced signaling pathways, which may lead to CXCL8 transcription. Regarding this objective, MAPKs are a hallmark of CysLT $_1$ R signaling, and it has been shown that PTP ϵ can inhibit ERK activation (Wabakken et al., 2002a; Toledano-Katchalski et al., 2003). The role of ERK1/2 was first studied in human monocytes using the MEK1 inhibitor PD98058 in LTD $_4$ -induced CXCL8 mRNA expression. As shown in Fig. 6A, the inhibition of ERK1/2 pathway resulted in the inhibition of LTD $_4$ -induced CXCL8 mRNA expression, confirming the role of this MAPK in CXCL8 expression. Next, to determine the function of PTP ϵ in LTD $_4$ -induced CXCL8 mRNA expression, human monocytes nucleofected with PTP ϵ -specific siRNAs were stimulated with LTD $_4$, and ERK1/2 phosphorylation was quantified. As illustrated by Western blot densitometry analysis, phosphorylation of both ERK1 and ERK2 is increased in human monocytes nucleofected with PTP ϵ -specific siRNAs compared with control siRNAs after LTD $_4$ stimulation (Fig. 6, B and C). In HEK-LT1 cells transiently expressing cyt-PTP ϵ , a significant decrease in both ERK1 and ERK2 phosphorylation (Fig. 6, D and E) was observed, whereas p38 and c-Jun-N-terminal kinase were not affected (Supplemental Fig. 7).

To identify ERK as a substrate of PTP ϵ , substrate-trapping constructs of the phosphatase—including an aspartic acid mutation to an alanine residue—were used. Since the tyrosine dephosphorylation process is quite rapid, these constructions allow the coimmunoprecipitation of phosphatase substrates, as the substrate will detach much more slowly from the catalytic pocket of the phosphatase. In HEK-LT1 cells, transiently transfected with substrate-trapping mutants (cyt-PTP ϵ _D245A-FLAG or RPTP ϵ _D303A-FLAG), ERK1/2 coimmunoprecipitated with the cyt-PTP ϵ isoform only upon LTD $_4$ stimulation (Fig. 6F), thus indicating that the two proteins could be found in a LTD $_4$ stimulation-dependent signaling complex. No consistent association was found with RPTP ϵ . It may then be assumed that dephosphorylation of ERK by cyt-PTP ϵ would lead to lower CXCL8 production after LTD $_4$ stimulation.

Discussion

Reversible phosphorylation of proteins is one of the main mechanisms that regulate cellular processes. Although this process relies on a balance, where PTPs and protein tyrosine kinases are the main characters, the latter have traditionally received much more attention; however, several studies have led to the recognition that PTPs could play essential roles

in the phosphorylation equilibrium in cells, as well as in physiologic processes (Fischer et al., 1991), and, therefore, be involved in numerous diseases (Hendriks et al., 2013).

The present work provides evidence that PTP ϵ and CysLT $_1$ R colocalize in human primary monocytes and in an ectopic expression model using HEK-293 cells. Furthermore, expression of PTP ϵ in HEK-LT1 cells has a concentration-dependent inhibitory effect on CysLT $_1$ R signaling in terms of LTD $_4$ -induced CXCL8 promoter transactivation, expression, and secretion. Conversely, transfection of human primary monocytes with PTP ϵ -specific siRNAs resulted in reduced PTP ϵ expression and concomitantly enhanced CXCL8 expression. Hence, our data suggest that PTP ϵ is a signaling partner of CysLT $_1$ R, providing negative regulation of its signal transduction.

Admittedly, PTPs are implicated in GPCR signal transduction; however, direct association with GPCRs has not often been reported. SHP-1 was shown, by coimmunoprecipitation, to associate with the chemokine receptor CXCR4 upon CXCL12 stimulation in MOLT4 cells (Vila-Coro et al., 1999) and with the somatostatin receptor SST2 in Chinese hamster ovary cells (Lopez et al., 1997). SHP-2, on the other hand, colocalizes with the gastrin receptor CCK2 (Vatinel et al., 2006), the orexin neuropeptide OX1 receptor (Voisin et al., 2008; El Firar et al., 2009), the β_2 -adrenergic receptor (Duchene et al., 2002), and the SST2 receptor (Ferjoux et al., 2003) in various cell types. ITIM (S/I/V/LxYxxI/V/L) and ITSM (TxYxxV/I) motifs within the amino acid sequences of the CCK2 and OX1 receptors were associated with the direct recruitment of both SHP-1 and SHP-2. As PTP ϵ was first identified as a partner of CysLT $_1$ R using the third intracellular loop of CysLT $_1$ R as a bait in a two-hybrid assay, this strongly suggested that this interaction is also direct; however, the region of interaction is not known at this time, as neither ITIM nor ITSM motifs are found in the receptor.

Given that we identified an interaction between CysLT $_1$ R and PTP ϵ in a two-hybrid assay, we used in cellulo techniques to confirm the association. At first, colocalization studies using confocal microscopy imaging allowed us to detect a spatial overlap between CysLT $_1$ R and PTP ϵ in HEK-LT1 cells, as well as in human primary monocytes; however, we could not discriminate between the multiple isoforms in the primary cells. Colocalization was further confirmed in HEK-293 cells using bimolecular fluorescence complementation, a protein reconstruction technique, in which fluorescence could be seen only if CysLT $_1$ R and PTP ϵ (each tagged with half of Venus) were close enough to reconstitute the fluorescent protein. pbFRET, which further allowed confirmation of their proximity using primary cells. Lastly, BRET² was used to characterize and quantify CysLT $_1$ R and PTP ϵ interaction in live cells. As shown with saturation curves, both RPTP ϵ and cyt-PTP ϵ colocalized with CysLT $_1$ R, although cyt-PTP ϵ had a stronger association with the receptor. By its cytoplasmic localization, however, cyt-PTP ϵ may simply have a greater ease of movement to associate with CysLT $_1$ R compared with RPTP ϵ , which is found mainly at the plasma membrane. These results support previous data on PTP ϵ cellular distribution since only RPTP ϵ possesses a membrane localization sequence (Gil-Henn et al., 2000).

As a second part of this study, we investigated the role of PTP ϵ in LTD $_4$ -induced CysLT $_1$ R signaling. Since we had previously shown that CysLT $_1$ R signaling induced CXCL8 promoter transactivation through activator protein-1 (AP-1)

Fig. 6. PTP ϵ reduces MAPK ERK1/2 activation. (A) Human primary monocytes were either untreated or pretreated for 15 minutes with PD98059 (10 μ M) and stimulated with either EtOH (vehicle) or 1 pM LTD₄, where indicated, for 6 hours and CXCL8 mRNA expression was quantified by RT-qPCR as $2^{\Delta\Delta CT}$ over RPL13A mRNA expression ($n = 10$). (B: ERK1, C: ERK2) 48 hours after nucleofection with the corresponding siRNAs (dotted line: siCTRL, black line: siPTP ϵ), human primary monocytes were stimulated with EtOH or LTD₄ (10 nM) for the indicated periods, and densitometry analysis was used to quantify p-ERK expression corrected with total ERK expression. A representative Western blot is shown ($n = 6$). (D: ERK1, E: ERK2) HEK-LT1 cells were transiently transfected with pcDNA3 (dotted line), cyt-PTP ϵ (gray line), or RPTP ϵ (black line) and then were stimulated with EtOH or LTD₄ (10 nM) for the indicated times. Western blots were performed, and densitometry analysis was used to quantify p-ERK expression corrected with total ERK expression. A representative Western blot is shown ($n = 6$). (F) HEK-LT1 cells were transiently transfected with FLAG-tagged PTP ϵ substrate-trapping mutants constructs—cyt-PTP ϵ _D245A-FLAG (gray line) or RPTP ϵ _D303A-FLAG (black line)—for 48 hours and were stimulated with EtOH or LTD₄ (10 nM) for the indicated time. Whole-cell lysates were immunoprecipitated with FLAG antibody, and immunoprecipitates were used for Western blots to quantify PTP ϵ and ERK total protein expression. A representative Western blot is shown ($n = 4$). Data are expressed as mean $\pm$ S.E.M. * $P < 0.05$; ** $P < 0.005$, by (A) paired t test, (B, C, and F) two-way ANOVA with Sidak's multiple comparisons post-test to compare cyt-PTP ϵ or RPTP ϵ with control (pcDNA3) or the siPTP ϵ with siCTRL and (D and E) two-way ANOVA with Dunnett's multiple comparisons post-test to compare cyt-PTP ϵ or RPTP ϵ with control (pcDNA3).

and NF- κ B transcription factors (Thompson et al., 2006), we used this model to study the implication of both cyt-PTP ϵ and RPTP ϵ in the chemokine's expression.

CXCL8 is associated with multiple inflammatory diseases, including arthritis (Troughton et al., 1996) and psoriasis (Zalewska et al., 2006), as well as the pathophysiology of

numerous lung diseases (Mukaida, 2003). CXCL8 is also a cardinal feature of severe asthma (Silvestri et al., 2006; Turkeli et al., 2015). Accordingly, higher CXCL8 promoter activity was quantified in asthmatic patients in whom symptoms were poorly controlled (John et al., 2009). Because the AP-1 transcription factor is involved in LTD₄-induced CXCL8

promoter activation (Thompson et al., 2006), we examined the role of the upstream kinases, MAPK ERK1/2 (Karin, 1995), since they were known to be direct substrates of cyt-PTP ϵ (Toledano-Katchalski et al., 2003). Moreover, higher ERK1/2 phosphorylation levels were detected in severe asthmatic subjects (Liu et al., 2008; Chang et al., 2012). Our results showed downregulation of LTD $_4$ -induced CXCL8 promoter transactivation, expression and protein secretion in HEK-LT1 cells expressing PTP ϵ , and, inversely, greater expression of the chemokine in human primary monocytes transfected with PTP ϵ -specific siRNAs. Basal expression of CXCL8 was not affected in transfected monocytes, indicating the necessity for phosphatase activation. Accordingly, a time-dependent association between ERK1/2 and cyt-PTP ϵ upon LTD $_4$ stimulation was found in HEK-LT1 cells transiently expressing the substrate-trapping mutant of the phosphatase. The kinase could not be consistently coimmunoprecipitated with the RPTP ϵ substrate-trapping mutant, thus corroborating our results, which show a decrease of LTD $_4$ -induced ERK1/2 phosphorylation only in the presence of cyt-PTP ϵ . In addition, decreased expression PTP ϵ led to greater LTD $_4$ -induced ERK1/2 phosphorylation and CXCL8 expression in human primary monocytes, although we were not able to differentially downregulate the two isoforms.

Although the siRNAs used in human monocytes were not isoform-specific, our experiments with cells transiently expressing cyt-PTP ϵ or RPTP ϵ are indicative of differential PTP ϵ isoform functions. Whereas both RPTP ϵ and cyt-PTP ϵ inhibit LTD $_4$ -induced CXCL8 expression, apparently, they do not exert their activity via the same substrates. Several signaling pathways have been shown to be differentially regulated by PTP ϵ isoforms: The insulin receptor and leptin receptor downstream events are decreased by RPTP ϵ through AKT (Møller et al., 1995; Lacasa et al., 2005; Nakagawa et al., 2005) and JAK2 dephosphorylation (Roussio-Noori et al., 2011), respectively. RPTP ϵ is involved in the maintenance of the mammary tumor phenotype induced by Neu or Ras through Src activation (Elson, 1999; Gil-Henn and Elson, 2003; Berman-Golan and Elson, 2007). Moreover, in addition to Src, RPTP ϵ activates Fyn and Yes (Granot-Attas and Elson, 2004). Also, RPTP ϵ is involved in myelination of the optic nerve. Cyt-PTP ϵ , on the other hand, has a role in axon myelination of the sciatic nerve and in the voltage-gated K $^+$ channels' Kv1.5 and Kv2.1 activity (Peretz et al., 2000; Tiran et al., 2003, 2006). Cyt-PTP ϵ is involved in inhibiting JAK/STAT signaling by dephosphorylation of STAT1 and STAT3 in murine M1 cells (Tanuma et al., 2000, 2001, 2003). Also, osteoclast function is influenced by the presence of cyt-PTP ϵ as the phosphatase allows a functional rearrangement of podosomes through Src activation (Chiusaroli et al., 2004; Granot-Attas et al., 2009; Finkelshtein et al., 2014).

In contrast to published data indicating that PTP ϵ isoforms are expressed in a nonoverlapping expression pattern in most murine cell types (Elson and Leder, 1995; Gil-Henn et al., 2000), our results indicate that both cyt-PTP ϵ and RPTP ϵ are expressed in human primary monocytes. Expression of both isoforms has also been identified in human mast cells (Akimoto et al., 2009), indicating a possible divergence in PTP ϵ regulation of expression between species. Moreover, PTP ϵ is highly expressed in immune cells, particularly in peripheral blood lymphocytes, and in lungs (Tanuma et al.,

1999; Wabakken et al., 2002a), where a considerable population of immune cells is found in asthmatic patients. In addition, PTP ϵ -deficient macrophages show a deficit in oxidative burst and cytokine production (IL-10 and TNF α) (Sully et al., 2001). PTP ϵ negatively regulates mast cell activation (Akimoto et al., 2009) and negatively regulates cytokine production by targeting STAT1 and STAT3 in IL-6 and IL-10 signaling (Tanuma et al., 2001). Thus, PTP ϵ is actively involved in immune signaling pathways, and our results add support to the interpretation of a regulatory/inhibitory role of PTP ϵ in immune signaling. Interestingly, a polymorphism in the *PTPRE* gene (*PTPRE*_rs7081735) has been associated with allergic asthma (Tremblay et al., 2008), potentially making its interaction with CysLT $_1$ R even more significant. Likewise, LTD $_4$ has also been found to induce the production of numerous cytokines, chemokines, and growth factors in human monocytes (Rola-Pleszczynski and Stankova, 2007). Hence, PTP ϵ may also be implicated in regulating the production of these inflammatory molecules as well.

Our results suggest that PTP ϵ may be important in inflammatory processes modulated by cys-LTs, and an understanding of the interaction of CysLT $_1$ R and PTP ϵ , both being associated with asthma, may further define their involvement in this disease and potentially provide a novel therapeutic target.

Acknowledgments

We thank Geneviève Hamel-Côté for technical support and for pertinent discussions throughout the course of this work.

Authorship Contributions

Participated in research design: Lapointe, Rola-Pleszczynski, Stankova

Conducted experiments: Lapointe, Turcotte, Véronneau

Performed data analysis: Lapointe, Rola-Pleszczynski, Stankova

Wrote or contributed to the writing of the manuscript: Lapointe, Rola-Pleszczynski, Stankova

References

- Akimoto M, Mishra K, Lim KT, Tani N, Hisanaga SI, Katagiri T, Elson A, Mizuno K, and Yakura H (2009) Protein tyrosine phosphatase epsilon is a negative regulator of Fc ϵ RI-mediated mast cell responses. *Scand J Immunol* **69**:401–411.
- Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, and Mustelin T (2004) Protein tyrosine phosphatases in the human genome. *Cell* **117**:699–711.
- Andersen JN, Elson A, Lammers R, Rømer J, Clausen JT, Møller KB, and Møller NP (2001) Comparative study of protein tyrosine phosphatase-epsilon isoforms: membrane localization confers specificity in cellular signalling. *Biochem J* **354**:581–590.
- Bakken T, He M, and Cannon ML (2010) The phosphatase Shp2 is required for signaling by the Kaposi's sarcoma-associated herpesvirus viral GPCR in primary endothelial cells. *Virology* **397**:379–388.
- Berman-Golan D and Elson A (2007) Neu-mediated phosphorylation of protein tyrosine phosphatase epsilon is critical for activation of Src in mammary tumor cells. *Oncogene* **26**:7028–7037.
- Boudaud M, Turcotte S, Stankova J, and Rola-Pleszczynski M (2018) IL-33 upregulates cysteinyl leukotriene receptor type 1 expression in human peripheral blood CD4 $^+$ T lymphocytes. *J Immunol* **201**:2787–2798.
- Böyum A (1968) Separation of leukocytes from blood and bone marrow. Introduction. *Scand J Clin Lab Invest Suppl* **97**:1.
- Chang PJ, Bhavsar PK, Michaeloudes C, Khorasani N, and Chung KF (2012) Corticosteroid insensitivity of chemokine expression in airway smooth muscle of patients with severe asthma. *J Allergy Clin Immunol* **130** (4):877–885.e5.
- Chiusaroli R, Knobler H, Luxenburg C, Sanjay A, Granot-Attas S, Tiran Z, Miyazaki T, Harmelin A, Baron R, and Elson A (2004) Tyrosine phosphatase epsilon is a positive regulator of osteoclast function in vitro and in vivo. *Mol Biol Cell* **15**:234–244.
- Duchene J, Schanstra JP, Pecher C, Pizard A, Susini C, Esteve JP, Bascands JL, and Girolami JP (2002) A novel protein-protein interaction between a G protein-coupled receptor and the phosphatase SHP-2 is involved in bradykinin-induced inhibition of cell proliferation. *J Biol Chem* **277**:40375–40383.
- Dussault AA and Pouliot M (2006) Rapid and simple comparison of messenger RNA levels using real-time PCR. *Biol Proced Online* **8**:1–10.

- El Firar A, Voisin T, Rouyer-Fessard C, Ostuni MA, Couvineau A, and Laburthe M (2009) Discovery of a functional immunoreceptor tyrosine-based switch motif in a 7-transmembrane-spanning receptor: role in the orexin receptor OX1R-driven apoptosis. *FASEB J* 23:4069–4080.
- Elson A (1999) Protein tyrosine phosphatase epsilon increases the risk of mammary hyperplasia and mammary tumors in transgenic mice. *Oncogene* 18:7535–7542.
- Elson A and Leder P (1995) Identification of a cytoplasmic, phorbol ester-inducible isoform of protein tyrosine phosphatase epsilon. *Proc Natl Acad Sci USA* 92:12235–12239.
- Ferjoux G, Lopez F, Esteve JP, Ferrand A, Vivier E, Vely F, Saint-Laurent N, Pradayrol L, Buscail L, and Susini C (2003) Critical role of Src and SHP-2 in sst2 somatostatin receptor-mediated activation of SHP-1 and inhibition of cell proliferation. *Mol Biol Cell* 14:3911–3928.
- Finkelshtein E, Lotinun S, Levy-Apter E, Arman E, den Hertog J, Baron R, and Elson A (2014) Protein tyrosine phosphatases ϵ and α perform nonredundant roles in osteoclasts. *Mol Biol Cell* 25:1808–1818.
- Fischer EH, Charbonneau H, and Tonks NK (1991) Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* 253:401–406.
- Florio T, Yao H, Carey KD, Dillon TJ, and Stork PJ (1999) Somatostatin activation of mitogen-activated protein kinase via somatostatin receptor 1 (SSTR1). *Mol Endocrinol* 13:24–37.
- Gil-Henn H and Elson A (2003) Tyrosine phosphatase-epsilon activates Src and supports the transformed phenotype of Neu-induced mammary tumor cells. *J Biol Chem* 278:15579–15586.
- Gil-Henn H, Volohonsky G, and Elson A (2001) Regulation of protein-tyrosine phosphatases alpha and epsilon by calpain-mediated proteolytic cleavage. *J Biol Chem* 276:31772–31779.
- Gil-Henn H, Volohonsky G, Toledano-Katchalski H, Gandre S, and Elson A (2000) Generation of novel cytoplasmic forms of protein tyrosine phosphatase epsilon by proteolytic processing and translational control. *Oncogene* 19:4375–4384.
- Granot-Attas S and Elson A (2004) Protein tyrosine phosphatase epsilon activates Yes and Fyn in Neu-induced mammary tumor cells. *Exp Cell Res* 294:236–243.
- Granot-Attas S, Luxenburg C, Finkelshtein E, and Elson A (2009) Protein tyrosine phosphatase epsilon regulates integrin-mediated podosome stability in osteoclasts by activating Src. *Mol Biol Cell* 20:4324–4334.
- Hamel-Côté G, Gendron D, Rola-Pleszczynski M, and Stankova J (2017) Regulation of platelet-activating factor-mediated protein tyrosine phosphatase 1B activation by a Janus kinase 2/calpain pathway. *PLoS One* 12:e0180336.
- Hay DW, Torphy TJ, and Udem BJ (1995) Cysteinyl leukotrienes in asthma: old mediators up to new tricks. *Trends Pharmacol Sci* 16:304–309.
- Hendriks WJ, Elson A, Harroch S, Pulido R, Stoker A, and den Hertog J (2013) Protein tyrosine phosphatases in health and disease. *FEBS J* 280:708–730.
- Ishikawa-Ankerhold HC, Ankerhold R, and Drummen GP (2012) Advanced fluorescence microscopy techniques—FRAP, FLIP, FLAP, FRET and FLIM. *Molecules* 17:4047–4132.
- John AE, Zhu YM, Brightling CE, Pang L, and Knox AJ (2009) Human airway smooth muscle cells from asthmatic individuals have CXCL8 hypersecretion due to increased NF-kappa B p65, C/EBP beta, and RNA polymerase II binding to the CXCL8 promoter. *J Immunol* 183:4682–4692.
- Karin M (1995) The regulation of AP-1 activity by mitogen-activated protein kinases. *J Biol Chem* 270:16483–16486.
- Kim CH, Qu CK, Hangoc G, Cooper S, Anzai N, Feng GS, and Broxmeyer HE (1999) Abnormal chemokine-induced responses of immature and mature hematopoietic cells from motheaten mice implicate the protein tyrosine phosphatase SHP-1 in chemokine responses. *J Exp Med* 190:681–690.
- Kraut-Cohen J, Muller WJ, and Elson A (2008) Protein-tyrosine phosphatase epsilon regulates Shc signaling in a kinase-specific manner: increasing coherence in tyrosine phosphatase signaling. *J Biol Chem* 283:4612–4621.
- Lacasa D, Boute N, and Issat T (2005) Interaction of the insulin receptor with the receptor-like protein tyrosine phosphatases PTPalpha and PTPepsilon in living cells. *Mol Pharmacol* 67:1206–1213.
- Lee JH, Choi SH, Lee BH, Hwang SH, Kim HJ, Rhee J, Chung C, and Nah SY (2013) Activation of lysophosphatidic acid receptor by gintonin inhibits Kv1.2 channel activity: involvement of tyrosine kinase and receptor protein tyrosine phosphatase α . *Neurosci Lett* 548:143–148.
- Levy-Apter E, Finkelshtein E, Vemulapalli V, Li SS, Bedford MT, and Elson A (2014) Adaptor protein GRB2 promotes Src tyrosine kinase activation and podosomal organization by protein-tyrosine phosphatase ϵ in osteoclasts. *J Biol Chem* 289:36048–36058.
- Liu W, Liang Q, Balzar S, Wenzel S, Gorska M, and Alam R (2008) Cell-specific activation profile of extracellular signal-regulated kinase 1/2, Jun N-terminal kinase, and p38 mitogen-activated protein kinases in asthmatic airways. *J Allergy Clin Immunol* 121 (4):893–902.e2.
- Lopez F, Estève JP, Buscail L, Delesque N, Saint-Laurent N, Théveniau M, Nahmias C, Vaysse N, and Susini C (1997) The tyrosine phosphatase SHP-1 associates with the sst2 somatostatin receptor and is an essential component of sst2-mediated inhibitory growth signaling. *J Biol Chem* 272:24448–24454.
- Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, Coulombe N, Abramovitz M, Figueroa DJ, Zeng Z, et al. (1999) Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 399:789–793.
- Marrero MB, Venema VJ, Ju H, Eaton DC, and Venema RC (1998) Regulation of angiotensin II-induced JAK2 tyrosine phosphorylation: roles of SHP-1 and SHP-2. *Am J Physiol* 275:C1216–C1223.
- Masoli M, Fabian D, Holt S, and Beasley R; Global Initiative for Asthma (GINA) Program (2004) The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 59:469–478.
- McCole DF, Truong A, Bunz M, and Barrett KE (2007) Consequences of direct versus indirect activation of epidermal growth factor receptor in intestinal epithelial cells are dictated by protein-tyrosine phosphatase 1B. *J Biol Chem* 282:13303–13315.
- Møller NP, Møller KB, Lammers R, Kharitonov A, Hoppe E, Wiberg FC, Sures I, and Ullrich A (1995) Selective down-regulation of the insulin receptor signal by protein-tyrosine phosphatases alpha and epsilon. *J Biol Chem* 270:23126–23131.
- Mukaida N (2003) Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol* 284:L566–L577.
- Nakagawa Y, Aoki N, Aoyama K, Shimizu H, Shimano H, Yamada N, and Miyazaki H (2005) Receptor-type protein tyrosine phosphatase epsilon (PTPepsilonM) is a negative regulator of insulin signaling in primary hepatocytes and liver. *Zool Sci* 22:169–175.
- Ngai J, Inngjerdigen M, Berge T, and Taskén K (2009) Interplay between the heterotrimeric G-protein subunits Galphq and Galphai2 sets the threshold for chemotaxis and TCR activation. *BMC Immunol* 10:27.
- Patrussi L, Capitani N, Cannizzaro E, Finetti F, Lucherini OM, Pelicci PG, and Baldari CT (2014) Negative regulation of chemokine receptor signaling and B-cell chemotaxis by p66Shc. *Cell Death Dis* 5:e1068.
- Peretz A, Gil-Henn H, Sobko A, Shinder V, Attali B, and Elson A (2000) Hypomyelination and increased activity of voltage-gated K(+) channels in mice lacking protein tyrosine phosphatase epsilon. *EMBO J* 19:4036–4045.
- Poisson C, Rollin S, Véronneau S, Bousquet SM, Larivière JF, Le Gouill C, Boulay G, Stankova J, and Rola-Pleszczynski M (2009) Caveolae facilitate but are not essential for platelet-activating factor-mediated calcium mobilization and extracellular signal-regulated kinase activation. *J Immunol* 183:2747–2757.
- Rabe KF, Adachi M, Lai CK, Soriano JB, Vermeire PA, Weiss KB, and Weiss ST (2004) Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *J Allergy Clin Immunol* 114:40–47.
- Rivard N, McKenzie FR, Brondello JM, and Pouyssegur J (1995) The phosphotyrosine phosphatase PTP1D, but not PTP1C, is an essential mediator of fibroblast proliferation induced by tyrosine kinase and G protein-coupled receptors. *J Biol Chem* 270:11017–11024.
- Rola-Pleszczynski M and Stankova J (2007) Cytokine-leukotriene receptor interactions. *Sci World J* 7:1348–1358.
- Roussou-Noori L, Knobler H, Levy-Apter E, Kuperman Y, Neufeld-Cohen A, Keshet Y, Akepati VR, Klinghoffer RA, Chen A, and Elson A (2011) Protein tyrosine phosphatase epsilon affects body weight by downregulating leptin signaling in a phosphorylation-dependent manner. *Cell Metab* 13:562–572.
- Silvestri M, Bontempelli M, Giacomelli M, Malerba M, Rossi GA, Di Stefano A, Rossi A, and Ricciardolo FL (2006) High serum levels of tumour necrosis factor-alpha and interleukin-8 in severe asthma: markers of systemic inflammation? *Clin Exp Allergy* 36:1373–1381.
- Sines T, Granot-Attas S, Weisman-Welcher S, and Elson A (2007) Association of tyrosine phosphatase epsilon with microtubules inhibits phosphatase activity and is regulated by the epidermal growth factor receptor. *Mol Cell Biol* 27:7102–7112.
- Sully V, Pownall S, Vincan E, Bassal S, Borowski AH, Hart PH, Rockman SP, and Phillips WA (2001) Functional abnormalities in protein tyrosine phosphatase epsilon-deficient macrophages. *Biochem Biophys Res Commun* 286:184–188.
- Tanuma N, Nakamura K, and Kikuchi K (1999) Distinct promoters control transmembrane and cytosolic protein tyrosine phosphatase epsilon expression during macrophage differentiation. *Eur J Biochem* 259:46–54.
- Tanuma N, Nakamura K, Shima H, and Kikuchi K (2000) Protein-tyrosine phosphatase PTPepsilon C inhibits Jak-STAT signaling and differentiation induced by interleukin-6 and leukemia inhibitory factor in M1 leukemia cells. *J Biol Chem* 275:28216–28221.
- Tanuma N, Shima H, Nakamura K, and Kikuchi K (2001) Protein tyrosine phosphatase epsilon C selectively inhibits interleukin-6- and interleukin-10-induced JAK-STAT signaling. *Blood* 98:3030–3034.
- Tanuma N, Shima H, Shimada S, and Kikuchi K (2003) Reduced tumorigenicity of murine leukemia cells expressing protein-tyrosine phosphatase, PTPepsilon C. *Oncogene* 22:1758–1762.
- Thivierge M, Stanková J, and Rola-Pleszczynski M (2001) IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages. *J Immunol* 167:2855–2860.
- Thompson C, Cloutier A, Bossé Y, Thivierge M, Gouill CL, Larivée P, McDonald PP, Stankova J, and Rola-Pleszczynski M (2006) CysLT1 receptor engagement induces activator protein-1- and NF-kappaB-dependent IL-8 expression. *Am J Respir Cell Mol Biol* 35:697–704.
- Tiran Z, Peretz A, Attali B, and Elson A (2003) Phosphorylation-dependent regulation of Kv2.1 channel activity at tyrosine 124 by Src and by protein-tyrosine phosphatase epsilon. *J Biol Chem* 278:17509–17514.
- Tiran Z, Peretz A, Sines T, Shinder V, Sap J, Attali B, and Elson A (2006) Tyrosine phosphatases epsilon and alpha perform specific and overlapping functions in regulation of voltage-gated potassium channels in Schwann cells. *Mol Biol Cell* 17:4330–4342.
- Tobin AB (2008) G-protein-coupled receptor phosphorylation: where, when and by whom. *Br J Pharmacol* 153 (Suppl 1):S167–S176.
- Toledano-Katchalski H and Elson A (1999) The transmembrane and cytoplasmic forms of protein tyrosine phosphatase epsilon physically associate with the adaptor molecule Grb2. *Oncogene* 18:5024–5031.
- Toledano-Katchalski H, Kraut J, Sines T, Granot-Attas S, Shohat G, Gil-Henn H, Yung Y, and Elson A (2003) Protein tyrosine phosphatase epsilon inhibits signaling by mitogen-activated protein kinases. *Mol Cancer Res* 1:541–550.
- Tremblay K, Lemire M, Potvin C, Tremblay A, Hunninghake GM, Raby BA, Hudson TJ, Perez-Iratxeta C, Andrade-Navarro MA, and Laprise C (2008) Genes to diseases (G2D) computational method to identify asthma candidate genes. *PLoS One* 3:e2907.
- Troughton PR, Platt R, Bird H, el-Manzalawi E, Bassiouni M, and Wright V (1996) Synovial fluid interleukin-8 and neutrophil function in rheumatoid arthritis and seronegative polyarthritis. *Br J Rheumatol* 35:1244–1251.
- Tsai W, Morielli AD, Cachero TG, and Peralta EG (1999) Receptor protein tyrosine phosphatase alpha participates in the m1 muscarinic acetylcholine

- receptor-dependent regulation of Kv1.2 channel activity. *EMBO J* **18**: 109–118.
- Turkeli A, Yilmaz O, Taneli F, Horasan GD, Kanik ET, Kizilkaya M, Gozukara C, and Yuksel H (2015) IL-5, IL-8 and MMP -9 levels in exhaled breath condensate of atopic and nonatopic asthmatic children. *Respir Med* **109**:680–688.
- Vatinel S, Ferrand A, Lopez F, Kowalski-Chauvel A, Estève JP, Fourmy D, Dufresne M, and Seva C (2006) An ITIM-like motif within the CCK2 receptor sequence required for interaction with SHP-2 and the activation of the AKT pathway. *Biochim Biophys Acta* **1763**:1098–1107.
- Vila-Coro AJ, Rodríguez-Frade JM, Martín De Ana A, Moreno-Ortiz MC, Martínez-A C, and Mellado M (1999) The chemokine SDF-1 α triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. *FASEB J* **13**:1699–1710.
- Vlahopoulos S, Boldogh I, Casola A, and Brasier AR (1999) Nuclear factor-kappaB-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation. *Blood* **94**:1878–1889.
- Voisin T, El Firar A, Rouyer-Fessard C, Gratio V, and Laburthe M (2008) A hallmark of immunoreceptor, the tyrosine-based inhibitory motif ITIM, is present in the G protein-coupled receptor OX1R for orexins and drives apoptosis: a novel mechanism. *FASEB J* **22**:1993–2002.
- Wabakken T, Hauge H, Finne EF, Wiedlocha A, and Aasheim H (2002) Expression of human protein tyrosine phosphatase epsilon in leucocytes: a potential ERK pathway-regulating phosphatase. *Scand J Immunol* **56**:195–203.
- Wabakken T, Hauge H, Funderud S, and Aasheim HC (2002) Characterization, expression and functional aspects of a novel protein tyrosine phosphatase epsilon isoform. *Scand J Immunol* **56**:276–285.
- Wang D, Paria BC, Zhang Q, Karpurapu M, Li Q, Gerthoffer WT, Nakaoka Y, and Rao GN (2009) A role for Gab1/SHP2 in thrombin activation of PAK1: gene transfer of kinase-dead PAK1 inhibits injury-induced restenosis. *Circ Res* **104**:1066–1075.
- Yu Z, Ahmad S, Schwartz JL, Banville D, and Shen SH (1997) Protein-tyrosine phosphatase SHP2 is positively linked to proteinase-activated receptor 2-mediated mitogenic pathway. *J Biol Chem* **272**:7519–7524.
- Zalewska A, Głowacka E, Wyczółkowska J, Tchórzewski H, Narbutt J, and Sysa-Jedrzejowska A (2006) Interleukin 6 and 8 levels in plasma and fibroblast cultures in psoriasis. *Mediators Inflamm* **2006**:81767.
- Zhu JW, Doan K, Park J, Chau AH, Zhang H, Lowell CA, and Weiss A (2011) Receptor-like tyrosine phosphatases CD45 and CD148 have distinct functions in chemoattractant-mediated neutrophil migration and response to *S. aureus*. *Immunity* **35**:757–769.

Address correspondence to: Jana Stankova, Université de Sherbrooke, 3001, 12e Avenue nord, Sherbrooke, QC J1H 5N4, Canada. E-mail: Jana.Stankova@USherbrooke.ca