Evidence for Regulation of the Tumor Necrosis Factor α-Convertase (TACE) by Protein-tyrosine Phosphatase PTPH1*

Yufang Zheng‡, Johannes Schlöndorff§, and Carl P. Blobel**

From the ‡Graduate Program in Physiology, Biophysics and Molecular Medicine, Weill Graduate School of Medical Science of Cornell University, New York, New York 10021, the §Tri-Institutional (Cornell University/Rockefeller University/Memorial Sloan-Kettering Cancer Center) M.D.-Ph.D. Training Program, New York, New York 10021, and the ¶Cellular Biochemistry and Biophysics Program, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received for publication, July 24, 2002
Published, JBC Papers in Press, August 30, 2002, DOI 10.1074/jbc.M207459200

Tumor necrosis factor α-convertase (TACE) is a metalloprotease-disintegrin involved in the ectodomain shedding of several proteins and is critical for proper murine development. TACE-mediated ectodomain shedding is regulated, and the cytoplasmic domain of TACE contains several potential signaling motifs, suggesting that this domain may play a role in regulating the metalloprotease activity. Here we report that the protein-tyrosine phosphatase PTPH1, which contains both a band 4.1 domain and a single PDZ domain, can interact with the cytoplasmic domain of TACE. The interaction was initially observed in a yeast two-hybrid screen and was confirmed using an in vitro binding assay and co-immunoprecipitations from eukaryotic cell extracts. The interaction is mediated via binding of the PDZ domain of PTPH1 to the COOH terminus of TACE. The latter represents a novel group I PDZ binding sequence characterized by a terminal cysteine residue. In co-expression experiments, significantly lower levels of TACE were observed in the presence of catalytically active forms of PTPH1 compared with catalytically inactive forms of PTPH1. Furthermore, phorbol ester-stimulated shedding of the TACE substrate tumor necrosis factor α was decreased in cells expressing catalytically active PTPH1 compared with inactive PTPH1. Taken together, these results suggest that PTPH1 may be a negative regulator of TACE levels and function, and thus provide the first evidence for the regulation of TACE through a cytoplasmic protein.

Ectodomain shedding is a process in which transmembrane proteins are proteolytically cleaved to release their extracellular domain or ectodomain from the membrane. Numerous proteins, including growth factors, cytokines, growth factor receptors, and adhesion molecules, are known to undergo ectodomain shedding, and this process can have a significant impact on the biological function of its target proteins (reviewed in Refs. 1–4). Tumor necrosis factor α-convertase (TACE),1 responsible for the processing of proTNF-α, was the first metalloprotease sheddase to be cloned and identified (5, 6).

TACE (ADAM17) is a member of the ADAM (α disintegrin and metalloprotease) family of metalloproteases, also known as metalloprotease-disintegrins, or MDC (metalloprotease, disintegrin, cysteine-rich) proteins (reviewed in Refs. 7–9). In addition to processing TNF-α, TACE has now been implicated as the ectodomain sheddase of transforming growth factor-α (TGF-α), L-selectin, TNF receptor I and II (10), interleukin-1 receptor II (11), HER4 (12), and as an amyloid precursor protein α-secretase (13). TACE may also be involved in the processing of Notch (14). TACE-deficient mice have several defects reminiscent of those seen in animals lacking either TGF-α or the epidermal growth factor receptor, and die in late embryogenesis or shortly after birth (10). Thus, TACE appears to act as a sheddase with broad substrate specificity and plays an important role during development. Several additional members of the ADAM family of proteases appear to function as sheddases. Kuzbanian (Kuz/MADM/ADAM10) is shown to be involved in cleaving Notch (15), the Notch ligand Delta (16), and ephrin-A2 (17). In addition, Kuz has been reported to act as an amyloid precursor protein α-secretase (18). Four ADAMS (ADAM9, ADAM10, ADAM12, and ADAM17) have been implicated in the shedding of heparin-binding EGF-like growth factor (HB-EGF) (19–22), and ADAM19 has been suggested to have a role in shedding of neuregulin (23). Thus, TACE represents the founding member of a group of ADAM-type metalloproteases involved in ectodomain shedding.

Ectodomain shedding is a regulated process. Numerous stimuli, both pharmacological, such as phorbol esters and calcium ionophores, and physiological, such as ligands for the epidermal growth factor receptor and several G-protein-coupled receptors, are known to induce an increase in shedding (2, 3, 24–26). Analysis of Chinese hamster ovary cells that are unable to up-regulate shedding in response to several stimuli indicates that a common downstream pathway is activated by

1 The abbreviations used are: TACE, tumor necrosis factor α-convertase; TNF-α, tumor necrosis factor α; AP, alkaline phosphatase; PTP, protein-tyrosine phosphatase; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; ADAM, α disintegrin and metalloprotease; MDC, metalloprotease-disintegrin, cysteine-rich; WT, wild type; TGF, transforming growth factor; PBS, phosphate-buffered saline; PBST, phosphate-buffered saline with Tween 20; EGF, epidermal growth factor; HB, heparin-binding; MAP, mitogen-activated protein; DTT, dithiothreitol; MBP, maltose-binding protein.

‡ Supported in part by National Institutes of Health Medical Scientist Training Program Training Grant 5T32GM07739-17, the Louis and Rachel Rudin Family Foundation, and a Papanicolaou medical scientist fellowship.

§ To whom correspondence should be addressed: Cellular Biochemistry and Biophysics Program, Sloan-Kettering Inst., Memorial Sloan-Kettering Cancer Center, Box 368, 1275 York Ave., New York, NY 10021. Tel.: 212-639-2915; Fax: 212-717-3047; E-mail: c-blobel@ski.msckc.org.
several stimuli (25). Recent evidence indicates that the MAP kinase pathways may be a common effector (27–30). The factors involved in transducing signals from upstream components such as MAP kinases to lead to increased shedding remain unknown, although protein-tyrosine kinases are likely involved (29). Interestingly, in most cases the cytoplasmic domains of the substrates do not appear to be important in targeting the proteins for shedding (12, 31–34). In contrast, the cytoplasmic domains of TACE, KUZ, and ADAM9 contain potential signaling motifs. Protein kinase Cζ (PKζ) has been shown to directly bind to and phosphorylate the cytoplasmic domain of ADAM9, which also becomes phosphorylated when cells are treated with phorbol esters (19, 35). Overexpression of an active form of PKζ leads to increased HB-EGF shedding, possibly by activating ADAM9 (21), although ADAM9 is not essential for HB-EGF shedding in mouse embryonic fibroblasts (36). Furthermore, truncated forms of TACE, KUZ, and ADAM9 lacking their pro- and metalloprotease domains appear to act in a dominant negative fashion (15, 19, 37), and removing cytoplasmic sequences from the dominant negative forms of either KUZ or ADAM9 attenuated their activity (15, 19).

To identify molecules potentially involved in the regulation of TACE, we screened for proteins capable of interacting with its cytoplasmic domain. In addition to identifying MAD2 as a TACE binding partner (38), we report here that the protein-tyrosine phosphatase PTPH1 binds to the TACE cytoplasmic domain. The basis of this interaction and the potential role of PTPH1 in modulating TACE function are examined and discussed.

MATERIALS AND METHODS

Reagents—All chemicals were obtained from Sigma unless otherwise indicated. Pervernapate was generated immediately prior to each experiment by mixing sodium vanadate and hydrogen peroxide to a final concentration of 100 mM each. Protein A- and Protein G-Sepharose were purchased from Amersham Biosciences. Restriction enzymes and Taq polymerase were purchased from Roche Molecular Biochemicals. PCR Turbo DNA polymerase was obtained from Stratagene.

Expression Vectors—cDNA fragments encoding various segments of the TACE cytoplasmic domain (see Fig. 1C) were generated by PCR utilizing primers designed such that the 5′ ends contained an EcoRI restriction site and the 3′ ends contained a stop codon and SstI restriction site. The resulting fragments were cloned into the pGEM3 vector (Clontech) for use in the yeast two-hybrid system and into pGEX-4T-1 (Amersham Biosciences) for the generation of bacterially expressed GST fusion proteins. Point mutations in the TACE cytoplasmic domain were generated by incorporating the desired mutation into the 3′ PCR primer. Plasmids encoding the GAL4 DNA-binding domain fused to various other ADAM cytoplasmic tails have been described previously (39). All of the resulting plasmids were sequenced (BioResource Center, Cornell University, Ithaca, NY) to rule out the possibility of unwanted PCR-induced mutations. The full-length TACE cDNA construct was generated by PCR and ligated into pcDNA3.1/Zeo (+) vector (Invitrogen) using the KpnI and BamHI sites. TACE Thr → Ala and TACE Cy5 → Ala were generated in the same fashion utilizing a 3′ PCR primer containing the desired mutation.

Similarly, PCR was utilized to generate fragments coding for various domains of PTPH1 utilizing a full-length PTPH1 cDNA as a template (see below). The resulting fragments were cloned in frame to the GAL4 activation domain of pGAD GH (Clontech), into pMAL-c2x (New England Biolabs), or into the mammalian expression vector pFLAG-CMV-2. The catalytically inactive Cys → Ser PTPH1 mutants were generated utilizing the QuickChange site-directed mutagenesis kit (Stratagene).

The cDNA for human TNF-α was kindly provided by Dr. M. Milla (University of Pennsylvania, Philadelphia, PA). The full-length TNF-α was excised by PCR utilizing primers designed such that the 5′ end contains a NheI site and a FLAG tag and the 3′ end contains a BglII site. The PCR product was introduced into the pPAtp5g vector (Genhunter Corp.) to produce a hTNF-α fusion protein with a FLAG tag on its NH2 terminus and an alkaline phosphatase (AP) protein and a Myc epitope and a His8 tag on its COOH terminus (Fig. 4A).

Yeast Two-hybrid Screen—The Clontech Matchmaker two-hybrid system was used to screen for proteins capable of interacting with the cytoplasmic domain of TACE as described previously (38). Briefly, the cytoplasmic tail of human TACE (amino acids 695–824), cloned into the pGBl9 vector, was used to screen a human HeLa cell cDNA library cloned into the pGAD GH vector (Clontech) using the yeast reporter strain AH109. Colonies capable of growth on medium were further assayed for lacZ gene expression using a colorimetric filter assay. The cDNA clones from yeast positive by both histidine prototrophy and β-galactosidase activity were isolated using protocols recommended by the manufacturer, transformed into XLI1 Blue Escherichia coli and sequenced (BioResource Center, Cornell University). The specificity of the interactions was tested by co-transforming potential positives with the control vectors pGBl9 and pLAM 5′ (Clontech), which encode for the GAL4 DNA-binding domain and the GAL4 DNA-binding domain fused to human lamin C, respectively. To map the domains involved in the interaction between TACE and PTPH1, various truncated forms of the TACE cytoplasmic domain or PTPH1 were used in the yeast two-hybrid system and into pGEX-4T-1 (see Fig. 1, C and D) expressed as the appropriate fusion proteins were introduced into the HFCe reporter strain and assayed by nutritional selection and β-galactosidase activity. All additional manipulations were performed using protocols recommended by the manufacturer (Clontech).

Library Screen—The PTPH1 probe (the entire ~3.0-kb cDNA insert isolated in the yeast two-hybrid screen) was 32P-labeled with the Prime-It II random primer labeling kit (Stratagene) followed by purification with a MicroSpin S-400 HR column (Amersham Biosciences). The resulting probe was used to screen an MDA-MB-468 Zap cDNA library (40). Initial positives were screened by PCR utilizing the T3 primer, which anneals to the 5′ multiple cloning sites in the λZap vector, and an antisense PTPH1 primer, to identify clones with the longest 5′ extension. Several clones predicted to contain the entire open reading frame of PTPH1 were plaque-isolated, and the cDNA insert excised using the ExAssist/SOLR system according to the protocol supplied by the manufacturer (Stratagene). One full-length clone was sequenced on both strands (BioResource Center, Cornell University).

Antibodies—The PTPH1 cDNA clone isolated in the yeast two-hybrid screen (encoding amino acids 402–913) was inserted into the pGEX-4T-3 vector (Amersham Biosciences), and the resulting plasmid was introduced into BL21 E. coli. The GST fusion protein was expressed and purified essentially as described (40) and used to immunize New Zealand White rabbits following established protocols (Covance Research Products, Denver, PA). The anti-TACE cytoplasmic and anti-TACE extracellular anti sera have been described elsewhere (40, 42, 43). Anti-FLAG M2 monoclonal antibody was purchased from Sigma, and the anti-TGF-β1-β1 G protein-binding (MBP) antibody from New England Biolabs. An anti-TNF-α polyclonal antibody was obtained from Endogen.

In Vitro Binding Assays—MBP-PTPH1 PDZ domain fusion protein was overexpressed in BL21 E. coli and purified following the manufacturer’s protocols (New England Biolabs). Cells overexpressing the various GST-TACE cytoplasmic fusion proteins were lysed in PBST (PBS, pH 7.4, 0.5% v/v Triton X-100, 0.5% v/v Na deoxycholate) and thawed cycles followed by a 13,000 × g spin for 20 min. Equal amounts of lysate were pre-incubated with glutathione-Sepharose 4B beads (Amersham Biosciences) in PBST for 20 min before the addition of purified MBP-PTPH1 PDZ to a final concentration of ~5 µg/ml. The solution was incubated at 4 °C for 2 h, and the beads were washed three times in PBST. Bound material was recovered by incubating in sample loading buffer at 95 °C for 5 min, separated by SDS-PAGE, and transferred to nitrocellulose (Schleicher & Schuell). Samples were analyzed by Ponceau staining, followed by Western blotting with an anti-MBP polyclonal antiserum.

Cell Culture, Transfection, and Immunoprecipitation—COS-7 cells (ATCC) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% (v/v) fetal bovine serum, 2 mM l-glutamine, 100 IU/ml penicillin G, and 100 µg/ml streptomycin. Cells seeded in six-well tissue culture plates (Falcon) were transfected with the desired expression plasmids using LipofectAMINE (Invitrogen). The transfection solution was removed after 6 h, and the cells were grown for 2 days in complete medium. In experiments examining the effect of phorbol 12-myristate 13-acetate (PMA), either PMA (to a final concentration of 25 nM) or vehicle was added 20 min prior to lysis. Cells were washed twice in PBS and lysed in 500 µl of lysis buffer (PBS, pH 7.4, with 1% (v/v) Triton X-100, 10 mM NaF, 1 mM Na2VO4, 1 mM EDTA, and 5 mM 1,10-phenanthroline)/well. Lysates from duplicate wells were pooled and cleared by centrifugation at 13,000 × g for 30 min. A small aliquot of lysate was set aside, mixed with 2× sample loading buffer containing 10 mM dithiothreitol (DTT)
and used to assay for protein expression by Western blot. The remaining lysate was incubated with 2 μg of anti-FLAG M2 monoclonal antibody (Sigma) and Protein A-Sepharose Fast Flow beads (Amersham Biosciences) for 2 h at 4 °C. After three 10-min washes in lysis buffer, the beads were incubated in sample loading buffer containing 10 mM DTT for 5 min at 95 °C. Samples were separated by 8% SDS-PAGE, transferred to nitrocellulose (Schleicher & Schuell), and analyzed by Western blotting essentially as in Ref. 41.

**TACE Expression and in Vivo Binding Assay**—Cos-7 cells were transiently transfected with pcDNA3.1-NTACE and either control vector (FLAG), pFLAG-MAD2, pFLAG-PTPH1 WT (PTPH1 WT), pFLAG-PTPH1 ΔC, or pFLAG-PTPH1 ΔΔC (PTPH1 ΔCAT), pFLAG-PTPH1 ΔΔ1 (PTPH1 ΔΔ1), or pFLAG-PTPH1 ΔΔ1 (PTPH1 ΔΔ1C→S) (Fig. 3A). Two days following transfection, the cells were lysed and the extracts were split into two aliquots. One aliquot was subjected to Western blot analysis with an anti-TACE antibody. The second aliquot was used to immunoprecipitate wild type or mutant forms of FLAG-tagged PTPH1 using an anti-FLAG antibody. The cells were lysed and the extracts were split into two aliquots. One aliquot was subjected to Western blot analysis with an anti-TACE antibody. The second aliquot was used to immunoprecipitate wild type or mutant forms of FLAG-tagged PTPH1 using an anti-FLAG antibody.

The cells were grown in OptiMEM for 1 h and then in OptiMEM with 25 ng/ml PMA for 3 days at 30 °C; growth under these conditions requires an interaction between the two fusion proteins. Identical results were obtained when the co-transformants were assayed for β-galactosidase activity. B: HFFc yeast were transformed with plasmids encoding the GAL4 activation domain fused to full-length PTPH1 and the GAL4 DNA binding domain fused to the cytoplasmic domains of various ADAMs. The ADAMs tested are MDC9 (ADAM9; meltrin μ), MADM (ADAM10; Kuz), meltrin α (ADAM12), MDC15 (ADAM15), TACE (ADAM17), and meltrin β (ADAM19). Colonies were streaked on to plates lacking histidine and grown for 3 days at 30 °C; growth under these conditions requires an interaction between the two fusion proteins. Identical results were obtained when the co-transformants were assayed for β-galactosidase activity. B: HFFc yeast were transformed with plasmids encoding the GAL4 activation domain fused to full-length PTPH1 and the GAL4 DNA binding domain fused to various segments of the TACE cytoplasmic domain. Numbers refer to the amino acids of TACE expressed in the fusion protein, C→V, C→A, and T→A refer to point mutants generated in the TACE cytoplasmic tail (see panel C). The ability of the two fusion proteins to interact was assessed by growing the transformants on histidine-free medium for 3 days at 30 °C. Colorimetric β-galactosidase assays confirmed these results (data not shown). C, summary of the TACE cytoplasmic domain constructs used and their ability to interact with PTPH1. The diagram represents the relative size and location of the TACE sequences fused to the GAL4 DNA binding domain. Numbers refer to amino acid residues. The right panel indicates whether the construct was capable of interacting with full-length PTPH1 based on both nutritional selection (B) and β-galactosidase assay (data not shown). D, summary of the PTPH1 constructs used and their ability to interact with the TACE cytoplasmic domain in the yeast two-hybrid system. A schematic of PTPH1 is shown indicating the position of the various domains, with the sequences that were fused to the GAL4 activation domain indicated below. Numbers refer to amino acid residues. The ability of the various fusion proteins to interact with the TACE cytoplasmic domain in the yeast two-hybrid system is indicated in the right column and reflect the results obtained from both growth on histidine-free medium and a colorimetric β-galactosidase assay.

**RESULTS**

PTPH1 Interacts with TACE via Its PDZ Domain in the Yeast Two-hybrid System—The cytoplasmic domain of TACE was used as bait in a yeast two-hybrid assay with a HeLa cDNA library. In addition to identifying MAD2 (38), a single clone encoding amino acids 402–913 of PTPH1 was found to interact with TACE based on both nutritional selection and β-galactosidase activity. This fragment of PTPH1 was not able to interact with the GAL4 DNA-binding domain alone or with a negative control protein (human lamin C fused to the GAL4 DNA-binding domain). To confirm that full-length PTPH1 can bind to the TACE cytoplasmic tail, a cDNA library generated from MDA-MB-468 cells (40) was screened and a cDNA encoding full-length PTPH1 was co-transformed with plasmids encoding full-length PTPH1 fused to the GAL4 activation domain and the GAL4 DNA binding domain fused to the cytoplasmic domains of various ADAMs. The ADAMs tested are MDC9 (ADAM9; meltrin μ), MADM (ADAM10; Kuz), meltrin α (ADAM12), MDC15 (ADAM15), TACE (ADAM17), and meltrin β (ADAM19). Colonies were streaked on to plates lacking histidine and grown for 3 days at 30 °C; growth under these conditions requires an interaction between the two fusion proteins. Identical results were obtained when the co-transformants were assayed for β-galactosidase activity. B: HFFc yeast were transformed with plasmids encoding the GAL4 activation domain fused to full-length PTPH1 and the GAL4 DNA binding domain fused to various segments of the TACE cytoplasmic domain. Numbers refer to the amino acids of TACE expressed in the fusion protein, C→V, C→A, and T→A refer to point mutants generated in the TACE cytoplasmic tail (see panel C). The ability of the two fusion proteins to interact was assessed by growing the transformants on histidine-free medium for 3 days at 30 °C. Colorimetric β-galactosidase assays confirmed these results (data not shown). C, summary of the TACE cytoplasmic domain constructs used and their ability to interact with PTPH1. The diagram represents the relative size and location of the TACE sequences fused to the GAL4 DNA binding domain. Numbers refer to amino acid residues. The right panel indicates whether the construct was capable of interacting with full-length PTPH1 based on both nutritional selection (B) and β-galactosidase assay (data not shown). D, summary of the PTPH1 constructs used and their ability to interact with the TACE cytoplasmic domain in the yeast two-hybrid system. A schematic of PTPH1 is shown indicating the position of the various domains, with the sequences that were fused to the GAL4 activation domain indicated below. Numbers refer to amino acid residues. The ability of the various fusion proteins to interact with the TACE cytoplasmic domain in the yeast two-hybrid system is indicated in the right column and reflect the results obtained from both growth on histidine-free medium and a colorimetric β-galactosidase assay.

To determine which sequences within the TACE cytoplasmic domain are important in mediating this interaction, plasmids encoding several truncated forms of the TACE cytotail were
introduced into yeast together with the full-length form of PTPH1. The resulting transformants were assayed both for growth on histidine-free medium and for β-galactosidase activity (Fig. 1, B and C). Based on these criteria, the COOH-terminal five amino acids of TACE (KETEC) are necessary and sufficient for interacting with PTPH1.

Complementary experiments utilizing truncated forms of PTPH1 indicate that the PDZ domain and a short amino-terminal spacer region (amino acids 402–605) are sufficient for interacting with the TACE cytoplasmic domain. The PDZ domains of several other proteins (termed class I PDZ domains) have been found to bind to the COOH terminus of their binding partners, specifically to a consensus sequence X-(S/T)-X-(I/V) (42). Although the TACE cytoplasm contains a threonine at the expected position, its carboxyl-terminal amino acid is a cysteine. To determine the relative importance of these amino acids of this region for the interaction with PTPH1, several point mutations in this sequence were generated. Mutating the carboxyl-terminal cysteine to an alanine, but not to a valine, abolished binding as assessed by nutritional and colorimetric assays. Furthermore, replacing the threonine with an alanine also abolished the interaction. These results indicate that the PDZ domain of PTPH1 binds to the carboxyl-terminal five amino acids of TACE and that these sequences are necessary and sufficient for the PTPH1-TACE interaction (Fig. 1, C and D).

**In Vitro Interaction of TACE with the PDZ Domain of PTPH1**—To establish that the interaction between the cytoplasmic domain of TACE and the PDZ domain of PTPH1 is direct, we utilized an in vitro binding assay. The PDZ domain of PTPH1 (amino acids 493–605) was expressed as a fusion protein with MBP. The resulting fusion protein (MBP-PTPH1 PDZ) was purified from bacteria and incubated with bacterial lysates containing either GST or GST fused to various segments of the TACE cytoplasmic tail. The GST fusion proteins were bound to glutathione-Sepharose beads, and their ability to co-precipitate MBP-PTPH1 PDZ was assessed by Western blot (Fig. 2). Similar to the interaction in the yeast two-hybrid system, the carboxyl-terminal five amino acids of TACE are necessary and sufficient for the interaction. Furthermore, these results indicate that the interaction between PTPH1 and TACE is direct.

**Interaction of TACE and PTPH1 in Eukaryotic Cells**—To confirm that the interaction between the cytoplasmic tail of TACE and PTPH1 can occur in a cellular context, several FLAG-tagged forms of PTPH1 (see Fig. 3A) were overexpressed in COS-7 cells together with a wild type TACE or two TACE mutants in which threonine 822 and cysteine 824 in the PDZ binding site are mutated to an alanine (TACE Thr → Ala and TACE Cys → Ala). Either mutant can abolish the interaction between TACE and PTPH1 in a yeast two-hybrid assay (see above). FLAG-tagged proteins were immunoprecipitated from cell lysates using an anti-FLAG monoclonal antibody. The presence of TACE proteins in the resulting immune complexes was assessed by Western blot utilizing an antiserum raised against the cytoplasmic domain of TACE. As shown in Fig. 3 (B and C), mouse TACE was co-immunoprecipitated to varying degrees by FLAG-tagged wild type PTPH1 as well as all mutant forms of PTPH1 tested. In contrast, a Thr → Ala mutation in the PDZ binding domain of TACE abrogated the interaction with all FLAG-tagged forms of PTPH1 tested here. The Cys → Ala mutation in the PDZ binding domain of TACE (TACE Cys → Ala) decreased the co-immunoprecipitation of all forms of PTPH1, but did not completely abolish binding. All TACE proteins were co-immunoprecipitated with FLAG-MAD2 as expected (32), and no TACE protein was immunoprecipitated when co-expressed with the pFLAG vector alone (Fig. 3B).

These data are consistent with the results of the yeast two-hybrid system and of the in vitro binding assay, confirming that the carboxyl-terminal sequence of TACE is necessary for PTPH1 binding.

In the experiments described above, TACE levels in cell lysates were always significantly lower in cells co-expressing catalytically active forms of PTPH1 (PTPH1 WT and PTPH1 ΔA1) compared with cells coexpressing inactive forms of PTPH1 (PTPH1 Cys → Ser, PTPH1 Δ4.1 Cys → Ser, PTPH1 ΔCAT), regardless of whether an interaction with the cytoplasmic tail of TACE could be detected. This finding suggests that the catalytic activity of overexpressed PTPH1 may affect TACE levels independently of an interaction with TACE.

To determine whether transfected PTPH1 also binds to endogenous TACE in COS-7 cells (Fig. 3D), FLAG-tagged PTPH1 lacking its catalytic domain (PTPH1 ΔCAT) and PTPH1 ΔA1 Cys → Ser were transfected into COS-7 cells. FLAG-tagged proteins were immunoprecipitated from cell lysates using an anti-FLAG monoclonal antibody, and the presence of endogenous monkey TACE proteins in the resulting immune complexes was assessed by Western blot with antibodies against the cytoplasmic domain of TACE. As shown in Fig. 3D, the pro-form and the mature form of monkey TACE both co-immunoprecipitated with the FLAG-tagged PTPH1 ΔCAT as well as PTPH1 ΔA1 Cys → Ser.
Effect of PTPH1 Expression on TNF-α Shedding—To address the possible role of PTH1 in regulating TACE function, we examined the effect of overexpressing PTPH1 on TNF-α shedding. For this purpose we developed a simple and quantitative assay for TNF-α shedding. As shown in Fig. 4A, a FLAG epitope was added to the NH₂ terminus of TNF-α, which is a type II transmembrane protein, and an alkaline phosphatase moiety followed by a Myc epitope and His₆ tag was attached to the COOH terminus of the TNF-α module. COS-7 cells transfected with alkaline phosphatase tagged TNF-α (TNF-α-AP) were incubated for 1 h in fresh medium in the presence of different concentrations of PMA (6.25, 12.5, 25, and 37.5 ng/ml), or as a control, with the carrier ethanol alone. TNF-α-AP released into the supernatant was purified on metal affinity beads via its His₆ tag. Bound TNF-α-AP was either subjected to SDS-PAGE and detected directly in the gel (Fig. 4B) or quantitated in a photometric alkaline phosphatase assay (Fig. 4C) as described under “Materials and Methods.” The shedding of TNF-α-AP in response to PMA was essentially identical to that wild type TNF-α in COS-7 cells (data not shown). Maximum shedding of TNF-α-AP was seen with as little as 12.5 ng/ml PMA, and the amount of
released TNF-α-AP was not significantly increased after stimulation with up to 37.5 ng/ml PMA (Fig. 4, B and C).

To quantitate how wild type or mutant forms of PTPH1 affect TNF-α shedding, COS-7 cells were transiently transfected with TNF-α-AP and either MAD2, PTPH1 WT, PTPH1 Cys → Ser, PTPH1 Δ4.1, or PTPH1 Δ4.1 Cys → Ser. The conditioned medium was collected after the cells were chased for 1 h in the presence or absence of 25 ng/ml PMA, which strongly stimulates TNF-α shedding (11). TNF-α-AP released into the conditioned medium was isolated and quantitated as described above (Fig. 5, A and B). These results suggest that catalytically active PTPH1 has a role in negatively regulating TACE function.

DISCUSSION

TACE is a prototypical protein ectodomain sheddase that has important roles in development and in the ectodomain release
Regulation of TACE by PTPH1

of proteins such as the pro-inflammatory cytokine TNF-α, the amyloid precursor protein, and ligands of the EGFR receptor. The release of TACE substrates such as TNF-α from the plasma membrane can be stimulated, for example by activators of protein kinase C or by inhibitors of protein-tyrosine phosphatases. Furthermore, the cytoplasmic domain of TACE contains several potential signaling motifs, including proline-rich Src homology 3 binding sites, suggesting that the function of TACE might be controlled through an interaction with cytoplasmic proteins. Here we report that the protein-tyrosine phosphatase PTPH1 can interact with the cytoplasmic domain of TACE, and demonstrate that PTPH1 may have a role in regulating the function of TACE.

PTPH1 is a member of a family of protein-tyrosine phosphatases characterized by the presence of an amino-terminal band 4.1 domain or ERM domain, a carboxyl-terminal phosphatase domain, and an intervening region containing one or more PDZ domains (43–45). PTPH1 expression has been observed in bone marrow, fetal liver, lymph nodes, and T cells (46). In Jurkat cells, PTPH1 is localized in the cytoplasm as well as in thin membrane projections on the cell surface. PTPH1 was first identified as a potential TACE-interacting protein in a yeast two-hybrid screen, and the interaction was confirmed by both in vitro and in vivo binding assays. In COS-7 cells, PTPH1 binds to both transfected mouse TACE and to the intrinsic monkey TACE. The interaction between TACE and PTPH1 is mediated through an interaction between the PDZ domain of PTPH1 and a carboxyl-terminal PDZ ligand domain in TACE (the carboxyl-terminal sequence KETEC). The presence of a threonine residue at the –2 position in the PDZ ligand domain of TACE is a common characteristic of ligands of group I PDZ domains (42). However, although the majority of group I PDZ domains contain V/I/L/M in the last position, TACE contains a carboxyl-terminal cysteine residue. Nevertheless, because cysteine is a non-polar amino acid, the PDZ binding sequence in TACE still fits in the general hydrophobic amino acid preference for group I PDZ domains (47).

The first indication for a role of PTPH1 in regulating the function of TACE came from experiments in which wild type and mutant forms of PTPH1 and TACE were co-expressed. The levels of transfected mouse TACE were consistently lower in cells expressing a catalytically inactive PTPH1 mutant (PTPH1 Cys → Ser). This effect did not depend on the presence of the band 4.1 domain, as a similar decrease in TACE levels was seen in cells expressing PTPH1 Δ4.1 compared with the catalytically inactive PTPH1 Δ4.1 Cys → Ser. Because we have been unable to detect tyrosine phosphorylation of the TACE cytoplasmic domain (data not shown), these results suggest that PTPH1 negatively regulates TACE levels through the dephosphorylation of other proteins.

PTPH1 has been shown to interact with two other proteins, 14-3-3β and valosin-containing protein (VCP, p97/CDC48). 14-3-3β, a member of the 14-3-3 family of phosphoserine-binding proteins involved in several signal transduction pathways, binds specifically to serine-phosphorylated PTPH1 (48). 14-3-3 family members have been implicated as scaffolding proteins involved in modulating the activity of several signaling pathways, including PKC and MAP kinase pathways (49–51). Because the MAP kinase pathway is involved in regulating release of the TACE substrate TGF-α (27), 14-3-3β could provide a link between PTPH1 and regulation of TACE levels or activity or both. The second currently known PTPH1 substrate, VCP, has a role in regulating cell cycle progression (52). In addition, other yet to be identified substrates of PTPH1 may have a role in regulating TACE levels or function. Unexpectedly, a TACE mutant that does not co-precipitate with PTPH1 responds to overexpression of wild type or mutant forms of PTPH1 in the same manner as wild type TACE. The regulation of TACE levels by PTPH1 thus does not require an interaction between these to binding partners. One possible explanation for this result is that PTPH1 and TACE may need to interact at physiological protein concentrations to regulate TACE, but that this requirement can be overcome by overexpression of the two binding partners.

To evaluate a potential functional regulation of intrinsic TACE by PTPH1, we tested how overexpressed wild type and mutant forms of PTPH1 affect the ectodomain shedding of TNF-α, a bona fide TACE substrate. We found a 25% decrease in phorbol ester-stimulated TNF-α release in cells expressing catalytically active wild type or mutant PTPH1 compared with cells expressing the corresponding catalytically inactive mutants (PTPH1 Cys → Ser or PTPH1 Δ4.1 Cys → Ser). Because Reddy et al. (11) have shown that the cytoplasmic domain of TACE is not required for the PMA-induced shedding of TNF-α, p75 tumor necrosis factor receptor, and interleukin-1 receptor II, we propose that the effect of PTPH1 on TNF-α shedding is likely the result of a change in TACE levels in the transfected cells as opposed to a direct regulation of TACE activity. Because a decrease in intrinsic TACE levels is difficult to ascertain in transiently transfected cells, where only a relatively small percentage of cells express the transfected proteins, further studies will be necessary to distinguish between these two possibilities.

Doedens and Black (53) have shown that cell surface TACE is down-regulated in response to prolonged stimulation with high doses of PMA (100 ng/ml). However, in the experiments presented here, lower doses of PMA (25 ng/ml) were used to stimulate shedding. Even prolonged exposure to these lower doses of PMA did not affect the levels of total endogenous COS-7 TACE, or the levels of overexpressed wild type or mutant forms of mouse TACE or PTPH1 (data not shown). Furthermore, we found no evidence for an effect of PMA on the interaction between TACE and PTPH1 (data not shown). Thus, our data provide no indication for a connection between the pathways responsible for down-regulation of TACE by PTPH1 and by PMA.

In summary, this study provides the first evidence for a regulation of TACE by interaction with a cytoplasmic protein, the protein-tyrosine phosphatase PTPH1. This interaction is mediated via the PDZ domain of PTPH1, which binds to the carboxyl terminus of TACE. Overexpression of PTPH1 decreases the levels of co-expressed mouse TACE, and also decreases the stimulated shedding of TNF-α, a TACE substrate. These results suggest that PTPH1 functions as a negative regulator of TACE levels as well as TACE function, most likely by dephosphorylating a yet to be identified effector. Further studies will focus on the mechanism underlying the regulation of TACE by PTPH1.

Acknowledgments—We are grateful to Dr. M. Milla for the human TNF-α cDNA clone and to Drs. L. Howard, K. Nelson, and G. Weskamp for generating various ADAM cytotail constructs.

REFERENCES

1. Ehlers, M. R., and Riordan, J. F. (1991) Biochemistry 30, 10065–10074
2. Massague, J., and Pandiella, A. (1993) Annu. Rev. Biochem. 62, 515–541
3. Hooper, N. M., Karran, E. H., and Turner, A. J. (1997) Biochem. J. 321, 265–279
4. Blobel, C. P. (2000) Curr. Opin. Cell Biol. 12, 606–612
5. Black, R., Rauch, C. T., Kozlosky, C. J., Peschon, J. J., Slack, J. L., Wolfson, M. F., Castner, B. J., Stocking, K. L., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K. A., Gerhart, M., Davis, R., Fiztaner, J. N., Johnson, R. S., Paxton, R. J., March, C. J., and Cerretti, D. P. (1997) Nature 385, 729–733
6. Moss, M. L., Jin, S.-L. C., Milla, M. E., Burkhart, W., Cartner, H. L., Chen, W.-J., Clay, W. C., Dubsbury, J. R., Hassler, D., Hoffman, C. R., Kost, T. A.,...
Regulation of TACE by PTPH1

Lambert, M. H., Lessnitzer, M. A., McCauley, P., McGeehan, G., Mitchell, J., Moyer, M., Piafel, G., Rocque, W., Overton, L. K., Schoenen, F., Seaton, T., Snell, L., Warner, J., Willard, D., and Becherer, J. D. (1997) Nature 385, 733–736

12. Rio, C., Buxbaum, J. D., Peschon, J. J., and Corfas, G. (2000) J. Biol. Chem. 275, 28828–28835

13. Fitzgerald, M. L., Wang, Z., Park, P. W., Murphy, G., and Bernfield, M. (2000) J. Cell Biol. 148, 811–824

14. Gunter, P., Oleszewski, M., Mechtersheimer, S., Agmon-Levin, N., Krauss, K., and Alvergro, P. (2000) J. Biol. Chem. 275, 15490–15497

15. Brabeck, C., Nophar, Y., Kemper, O., Engelmann, H., and Wallach, D. (1992) EMBO J. 11, 943–950

16. Brabeck, C., Varfolomeev, E. E., Batkin, M., and Wallach, D. (1994) J. Biol. Chem. 269, 32448–32456

17. Cheng, H. J., and Flanagan, J. G. (1994) Mol. Biol. Cell 5, 943–953

18. Arribas, J., Lopez-Casillas, F., and Massague, J. (1997) J. Biol. Chem. 272, 17160–17165

19. Roghani, M., Becherer, J. D., Mose, M. L., Atherton, R. E., Erdjument-Bromage, H., Arribas, J., Blackburn, R. K., Weskamp, G., Tempst, P., and Blobel, C. P. (1999) J. Biol. Chem. 274, 3531–3540

20. Weskamp, G., Cui, H., Brodie, T., Higashyama, S., Manova, K., Ludwig, T., and Blobel, C. (2002) Mol. Cell. Biol. 22, 1537–1544

21. Solomon, K. A., Pest, N., Wu, G., and Newton, R. C. (1999) J. Immunol. 163, 4105–4108

22. Nelson, K. K., Schlondorff, J., and Blobel, C. P. (1999) Biochem. J. 343, 673–680

23. Howard, L., Nelson, K. K., Maciewicz, R. A., and Blobel, C. P. (1999) J. Biol. Chem. 274, 31693–31699

24. Kratzschmar, J., Lum, L., and Blobel, C. P. (1996) J. Biol. Chem. 271, 4593–4596

25. Lum, L., Reid, M. S., and Blobel, C. P. (1998) J. Biol. Chem. 273, 26236–26247

26. Songyang, Z., Panning, A. S., Fu, C., Xu, J., Marfatia, S. M., Chiahti, A. H., Crompton, A., Chan, A. C., Anderson, J. M., and Cantley, L. C. (1997) Science 275, 73–77

27. Yang, Q., and Tonks, N. K. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5949–5953

28. Arimura, Y., Hinoda, Y., Itoh, F., Takekawa, M., Tsuchiaki, M., Adachi, M., Imai, K., and Yachi, A. (1992) Tumour Biol. 13, 180–186

29. Itoh, F., Ikuta, S., Hinoda, Y., Arimura, Y., Ohe, M., Adachi, M., Aiyama, T., Inazawa, J., Imai, K., and Yachi, A. (1993) Int. J. Cancer 55, 947–951

30. Gjerloff-Wingren, A., Saxena, M., Han, S., Wang, X., Alonso, A., Renedo, M., Oh, P., Williams, S., Schnitzer, J., and Mustelin, T. (2000) Eur. J. Immunol. 30, 2412–2421

31. Hung, A. Y., and Sheng, M. (2002) J. Biol. Chem. 277, 5599–5702

32. Zhang, S. H., Kobayashi, R., Graves, P. R., Pwnica-Worms, H., and Tonks, N. K. (1997) J. Biol. Chem. 272, 27281–27287

33. Balbino, P. D., and Hall, A. (1995) Curr. Biol. 5, 95–96

34. Baldin, V. (2000) Prog. Cell Cycle Res. 4, 49–69

35. Aitken, A., Jones, D., Soneji, Y., and Howell, S. (1995) Biochem. Soc. Trans. 23, 605–611

36. Zhang, S. H., Liu, J., Kobayashi, R., and Tonks, N. K. (1999) J. Biol. Chem. 274, 17806–17812

37. Doedens, J. R., and Black, R. A. (2000) J. Biol. Chem. 275, 14598–14607
Evidence for Regulation of the Tumor Necrosis Factor α-Convertase (TACE) by Protein-tyrosine Phosphatase PTPH1
Yufang Zheng, Johannes Schlöndorff and Carl P. Blobel

J. Biol. Chem. 2002, 277:42463-42470.
doi: 10.1074/jbc.M207459200 originally published online August 30, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M207459200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 53 references, 35 of which can be accessed free at http://www.jbc.org/content/277/45/42463.full.html#ref-list-1