Protein-tyrosine Phosphatase MEG2 Is Expressed by Human Neutrophils

LOCALIZATION TO THE PHAGOSOME AND ACTIVATION BY POLYPHOSPHOINOSITIDES*

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Signaling pathways involving reversible tyrosine phosphorylation are essential for neutrophil antimicrobial responses. Using reverse transcriptase PCR, expression of the protein-tyrosine phosphatase MEG2 by peripheral neutrophil polymorphonuclear leukocytes (PMN) was identified. Polyclonal antibodies against MEG2 were developed that confirmed expression of MEG2 protein by PMN. Through a combination of immunofluorescence and cell fractionation followed by immunoblotting, we determined that MEG2 is predominantly cytosolic with components present in secondary and tertiary granules and secretory vesicles. MEG2 activity, as determined by immunoprecipitation and in vitro phosphatase assays, is inhibited after exposure of cells to the particulate stimulant opsonized zymosan or to phorbol 12-myristate 13-acetate but largely unaffected by the chemoattractant N-formyl-methionyl-leucyl-phenylalanine. Studies using bacterially expressed glutathione S-transferase fusion protein indicate that cysteine 515 is essential for catalytic activity, whereas the noncatalytic (N-terminal) domain of MEG2 negatively regulates the enzymatic activity of the C-terminal phosphatase domain. The activity of MEG2 is enhanced by specific polyphosphoinositides with the order of potency being phosphatidylinositol (PI) 4,5-diphosphate > PI 3,4,5-triphosphate > PI 4-phosphate. MEG2 associates at an early stage with nascent phagosomes. Taken together, our results indicate that MEG2 is a polyphosphoinositide-activated tyrosine phosphatase that may be involved in signaling events regulating phagocytosis, an essential antimicrobial function in the innate immune response.

Neutrophils are an essential component of the innate immune system. To function in host defense, they have evolved a variety of mechanisms, including chemotaxis, phagocytosis, and intracellular killing of invading microbial pathogens. Paradoxically, unregulated activation of these responses can result in tissue injury, as is believed to occur in inflammatory disorders such as rheumatoid arthritis (1), inflammatory bowel disease (2, 3), ischemia-reperfusion (4, 5), and acute lung injury (6, 7). Leukocyte micridial responses must be precisely regulated by selective activation and rapid cessation of signaling cascades once the initial stimulus has been removed.

Signaling pathways involving tyrosine phosphorylation are pivotal to neutrophil antimicrobial responses, including adherence (8), chemotaxis (9), phagocytosis (10), and oxidant production (11–13). The level of tyrosine phosphorylation is regulated by the reciprocal activities of protein-tyrosine kinases (PTK) and protein-tyrosine phosphatases (PTP). Although PTK have been extensively studied, current knowledge of the identity and functional importance of PTP expressed in neutrophils is comparatively less. To date, CD45 (14–16), SHP-1 (17), SHP-2 (18), and CD148 (19) have been identified in neutrophils, but, by inference from studies in other cell types, additional PTP are likely to be expressed and participate in cell regulation (20).

During a search for supplementary PTP expressed by myeloid cells, we identified expression of PTP-MEG2 in neutrophils. MEG2 is a nonreceptor PTP originally cloned from human endothelial cell and megakaryocyte cell lines (21) and recently reported to be expressed in certain cell lines of hematopoietic lineage (22, 23). The C-terminal region of MEG2 contains the conserved PTP catalytic domain whereas the N-terminal domain has significant homology to yeast SEC14p (24) and cellular retinaldehyde binding protein (25, 26). Yeast SEC14p, an essential phosphatidylinositol (PI)phosphatidylcholine (PC) transfer protein, is involved in the transport of proteins from a late Golgi compartment to the plasma membrane and yeast vacuole (24, 27, 28). Cellular retinaldehyde

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† The abbreviations used are: PTK, protein-tyrosine kinase; CRALBP, cellular retinaldehyde binding protein; DOPC, dioleoyl-phosphatidylcholine; iMLP, N-formyl-methionyl-leucyl-phenylalanine; PBS, phosphate-buffered saline; FMA, phorbol 12-myristate 13-acetate; PMN, neutrophil polymorphonuclear leukocytes; PTP, protein-tyrosine phosphatase; SHP-1, SH2-containing phosphatase-1; PC, phosphatidylcholine; PI, phosphatidylinositol; PI 4-P, phosphatidylinositol 4-phosphate; PI 4,5-P₂, phosphatidylinositol 4,5-diphosphate; PI 3,4-P₂, phosphatidylinositol 3,4-diphosphate; MuLV, murine leukemia virus; DFF, diisopropylfluorophosphate; HA, hemagglutinin; RT, reverse transcriptase; GST, glutathione S-transferase; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; μNPP, p-nitrophenyl phosphate; Pipes, 1,4-piperazinediethanesulfonic acid; PI3K, phosphatidylinositol 3-kinase.
MEG2, a Polyphosphoinositide-activated Tyrosine Phosphatase

binding protein (CRALBP) serves as a carrier protein for metabolites of vitamin A that are intermediaries in the synthesis of rhodopsin (29, 30). The expression of MEG2 by peripheral blood neutrophils in concert with the homology of MEG2 with the lipid binding proteins SEC14p and CRALBP suggested to us a potential functional role for MEG2 in membrane transport or fusion events involved in myeloid cell function.

The objectives of the current study were to characterize the subcellular localization of MEG2 and to begin to elucidate its function in myeloid cells. Our results indicate that MEG2 is present in both the cytosol and granules and becomes incorporated into nascent phagosomes. The activity of MEG2 is enhanced by the polyphosphoinositides PI 4,5-P2, PI 3,4,5-P3, and PI 4-P. These observations suggest a role for MEG2 in the regulation of signaling pathways involved in phagocytosis.

**EXPERIMENTAL PROCEDURES**

**Materials**—MEG2-specific and degenerate primers were synthesized by General Synthesis and Diagnostics (Toronto, Ontario, Canada). Restriction enzymes and all other primers were purchased from Invitorgen (Burlington, Ontario, Canada). The TA Cloning kit and One-Shot kit were purchased from Invitrogen (San Diego, CA). MuLV reverse transcriptase and Ampli Tag DNA polymerase were purchased from PerkinElmer Life Sciences (Applied Biosystems, Mississauga, Ontario, Canada). The T7 Sequencing kit was purchased from Amersham Biosciences, Inc. (Bai D’Urfe, Quebec). Pfu polymerase was purchased from Stratagene (La Jolla, CA). Diisopropylfluorophosphate (DFP), lactoperoxidase, and horseradish peroxidase (HRP) were isolated from human peripheral blood neutrophils and HL-60 cells. Murine leukemia virus (MuLV) reverse transcriptase, and random hexamers from the Gene Amp RNA PCR kit were used for the reaction, which was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer/Cetus, Norwalk, CT). The PowerOne (Beckman Coulter) manufacturer’s instructions. Transcribed cDNA was then amplified using the degenerate primers for an initial five cycles with the following parameters: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. Another 30 cycles followed with denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. Transcribed cDNA was Amplified using MEG2 primer sets 1 and 2 for 35 cycles with denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 2 min. All components of the amplification mixtures were tested for contamination by running 35 PCR cycles in absence of the template RNA. Genomic DNA contamination was tested for by performing the RT-PCR in absence of the MuLV reverse transcriptase. All reactions were analyzed by agarose gel electrophoresis using ethidium bromide staining. Amplified cDNA sequence, a stop codon, and an 18-bp XhoI restriction site immediately followed by the first 18 bp of the coding MEG2 sequence was synthesized containing the final 15 bp of the coding MEG2 sequence, a stop codon, and an 18-bp restriction site.

**Reverse Transscriptase (RT) PCR—** cDNA was reverse-transcribed from total RNA isolated from human peripheral blood neutrophils and HL-60 cells. Murine leukemia virus (MuLV) reverse transcriptase, and random hexamers from the Gene Amp RNA PCR kit were used for the reaction, which was carried out in a DNA Thermal Cycler 480 (Perkin-Elmer/Cetus, Norwalk, CT). The PowerOne (Beckman Coulter) manufacturer’s instructions. Transcribed cDNA was then amplified using the degenerate primers for an initial five cycles with the following parameters: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. Another 30 cycles followed with denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min. Transcribed cDNA was Amplified using MEG2 primer sets 1 and 2 for 35 cycles with denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 2 min. All components of the amplification mixtures were tested for contamination by running 35 PCR cycles in absence of the template RNA. Genomic DNA contamination was tested for by performing the RT-PCR in absence of the MuLV reverse transcriptase. All reactions were analyzed by agarose gel electrophoresis using ethidium bromide staining. Amplified cDNA sequence, a stop codon, and an 18-bp restriction site immediately followed by the first 18 bp of the coding MEG2 sequence was synthesized containing the final 15 bp of the coding MEG2 sequence, a stop codon, and an 18-bp restriction site.

**Sequence of MEG2-hemagglutinin Fusion Protein Vector**—The cloning vector pGEX-4T1 (Amersham Biosciences, Inc.) was a gift from P. Majerus (Washington University School of Medicine). The MEG2 cDNA translation was then transferred to the pCDNA3 vector (Invitrogen). An XhoI restriction site was added to 5′-end of the MEG2 insert. A 5′-primer (CCCCCTCGAGATCCTGACGACAGTGCGATCTCCTCAACGGCGAG) and a 3′-primer (CTCAACGGCGAGGACCGGACG) were designed containing the final 24 bp of the coding MEG2 sequence, the hemagglutinin (HA) signal sequence, and a 1-bp XhoI restriction site. A 3′-primer (GCGGGATCCGGAGAGAGCCCGGAGCGGACGCG) was constructed containing the final 15 bp of the coding MEG2 sequence, a stop codon, and an 18-bp XhoI restriction site. Twenty-five cycles of the polymerase chain reaction (PCR) were performed with denaturation for 1 min at 94 °C, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min using Pfu polymerase. The PCR product was double-digested (BstEII, XhoI) and ligated into the MEG2-pCDNA3 construct in place of the existing BstEII-XhoI restriction site.

**GST-MEG2 Fusion Protein Constructs—** A 5′-primer (TTTTCGAAATTCTAGGCCGCGCCAAGCGGCGG) was synthesized containing an EcoRI site immediately followed by the first 18 bp of the coding MEG2 sequence. A 3′-primer (ACTTTCGAGATCCTGACGACAGTGCGATCTCCTCAACGGCGAGGAGAGCCCGGAGCGGACGCGACG) was synthesized containing the final 15 bp of the coding MEG2 sequence immediately followed by a stop codon and one EcoRI site. Twenty-five cycles of PCR were performed with denaturation for 1 min at 94 °C, annealing at 53 °C for 1 min, and extension at 72 °C for 1 min using Pfu polymerase and the MEG2-Bluescript construct as a template. The PCR product was digested with EcoRI and ligated into the EcoRI site of the pGEX-4T1 vector (Amersham Biosciences, Inc., Bai D’Urfe, Quebec). A catalytically inactive pGEX-MEG2 construct was constructed using a 5′-primer (CAGAAGGTACCTGCTCAGTCGATATGATGACTTCGAGAATCGCTTCAACGGCGAGGAGAGCCCGGAGCGGACGCGACG) and a 3′-primer (CTCAACGGCGAGGACCGGACG) which coted with a T to A substitution at position 1543 of the MEG2-coding cDNA sequence thus converting the cysteine 515 to serine. PCR was performed (as above) using a 5′-primer (TTTTCGAAATTCTAGGCCGCGCCAAGCGGCGG) targeting bp 919-936 and the 3′-primer (TGCGATTTGGAATTCGCGCGGCGCGGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCGCGGCGCGCG
MEG2, a Polyphosphoinositide-activated Tyrosine Phosphatase

pGEX-wild type MEG2 construct as template DNA. The BstEI-EcoRI region of the PCR product was used to replace the corresponding region in the pGEX-wild type MEG2 construct.

A pGEX construct containing the MEG2 noncatalytic domain and a histidine tag was developed using a 5′-primer (TTTTCGGAATTCATGGAGCCCGCGGACACCCG) containing a 5′-EcoRI and XhoI and a 3′-primer containing the corresponding sites of the pGEX-4T1 vector. A pGEX construct containing the MEG2 phosphatase domain and a hemagglutinin tag was developed using a 5′-primer (TTTTGCGGATCTGAGGACACCCGAGGAT) containing an EcoRI site immediately followed by the bp 859–873 of the coding MEG2 sequence. The 3′-primer (CCCTTCCTGATTGATGTGAGTGTGCTCCCGTGATAGCTAGGAGGACACCCG) contained the final 24 bp of the coding MEG2 sequence, the hemagglutinin (HA) sequence, a stop codon, and an XhoI restriction site. The pcDNA3-wild type MEG2-HA tag construct was used as a template. The PCR product was double-digested with EcoRI and XhoI and ligated with the corresponding sites of the pGEX-4T1 vector.

GST Fusion Protein Expression—Bacteria were cultured in LB media (catalyzed by 100 μg/ml ampicillin) and grown to an OD of 0.6–0.8. Samples were induced with 0.1 mM isopropyl-1-thio-β-galactopyranoside for 4 h, shaking at room temperature. Bacteria were centrifuged (3500 rpm in a Beckman GPR centrifuge), washed once in STE buffer (10 mM Tris, pH 5.0, 500 mM NaCl, 0.1% Tween 20) after which the antibody was eluted into 1-ml fractions using Gentle Elution Buffer (Pierce). After analyzing the samples at 280 nm, fractions with high protein concentration were pooled and dialyzed extensively against binding buffer.

SDS-PAGE and Immunoblotting—SDS-PAGE and immunoblotting were carried out as described previously (34). The Western blots were then developed using the enhanced chemiluminescence (ECL) detection system following the manufacturer's instructions (Amersham Biosciences, Inc.) overnight at 4 °C. The Western blots were stained twice in water and six times with assay buffer (25 mM Heps, 2.5 mM EDTA, 5 mM p-Nitrophenyl phosphate (pNPP) substrate, and 10 mM DTT. Samples were incubated for 30 min shaking at 37 °C, and absorbance was measured at 405 nm.

To test the effect of lipids on phosphatase activity, lipids were dissolved in methanol (10 μg/ml) and used to coat octyl-Sepharose beads (Amersham Biosciences, Inc.) overnight at 4 °C. The beads were washed twice in water and six times with assay buffer (25 mM Heps, 2.5 mM NaCl, 2.5 mM EDTA, pH 7.2) and incubated with 10 pmol of each fusion protein for 30 min at 37 °C. Subsequently, pNPP (10 mg/ml) and DTT (10 mM) were added and incubated for an additional 30 min at 37 °C. The supernatant was collected and the absorbance at 405 nm determined.

Phosphatidylcholine liposomes containing additional test lipids as a minor molar constituent (5% molar concentration) were prepared by evaporating lipid mixtures in a rotary evaporator followed by resuspension in an assay buffer composed of 30 mM Heps, pH 7.2, 100 mM NaCl, 1 mM EDTA. After one freeze-thaw cycle, the samples were extruded through a 400-nm filter and stored at 4 °C until use. The liposomes were labeled with [3H]Phosphatidyethanolamine to quantitatively concentrate.
To test the effect of the mixed liposome on MEG2 fusion protein function, an 80-μl reaction was prepared containing 25 μg/ml GST-MEG2 fusion protein, liposomes (250 μM lipid concentration), 40 mM Hepes, 100 mM NaCl, 1 mM EDTA, 10 mMDTT, 0.025% Triton X-100, and 0.02% P40 containing 10 μM µNPP and 10 mM DTT was added. Samples were incubated again at 37 °C and then collected, and the absorbance at 405 nm was measured as described above.

**RESULTS**

**PTP-MEG2 Is Expressed by Human Neutrophils**—During a search for PTP expressed by myeloid cells, expression of MEG2 in neutrophils was identified through an RT-PCR screen using degenerate primers targeted to the highly conserved catalytic domain of PTP. Of 150 clones that were sequenced, 98 shared complete sequence identity with four known PTP, including CD45 (n = 30), SHP-1 (n = 26), PTP1B (n = 22), and MEG2 (n = 20). The remainder of the clones had significant homology to ribosomal RNA (n = 34) or less than 1% homology with known proteins (n = 18) likely reflecting the degeneracy of the primers and the relatively low stringency of the initial PCR conditions. Expression of PTP-MEG2 by cell lines of hematopoietic lineage (21, 22), including the Jurkat T-cell line (23) has previously been reported. The expression of MEG2 by mature myeloid cells such as neutrophils has not been previously reported and accordingly, we focused our attention on this phosphatase.

Because our initial screening technique used degenerate primers, to confirm the expression of MEG2 in neutrophils an additional RT-PCR analysis was conducted with two additional sets of primers specific for MEG2. This additional analysis targeted two unique areas of MEG2: primer set 1 targeted base pairs 943–1124 of the MEG2 cDNA sequence and primer set 2 targeted a region in the C-terminal direction corresponding to base pairs 1402–1589. Bands of the predicted size (181 and 187 bp) resulted from RT-PCR amplification of total RNA from neutrophils (Fig. 1). A similar strategy demonstrated the expression of MEG2 mRNA by the myeloid cell line HL-60 (Fig. 1). DNA sequence analysis confirmed that these PCR products corresponded to MEG2 (not illustrated).

To determine if MEG2 protein was expressed in mature neutrophils, a polyclonal antibody was raised in rabbits against a GST-full-length MEG2 fusion protein. To test the specificity of this antibody (referred to as MEG2-whole molecule), Western analysis was performed on lysates of U937 cells transfected with HA-tagged MEG2. Fig. 2a illustrates that immune but not pre-immune serum recognized a protein of ~70 kDa corresponding to the predicted molecular mass of the recombinant fusion protein. Independent blotting with a monoclonal anti-HA antibody confirmed that the position of the band recognized by the immune serum corresponded to that of the epitope-tagged MEG2. Fig. 2b demonstrates that the affinity-purified immune serum recognized a single 68-kDa immunoreactive band in neutrophils corresponding to the predicted molecular mass of endogenous MEG2. Immunodetection of this band could be competed by the immunizing protein, confirming the specificity of the antibodies. A second polyclonal antipeptide antibody was raised against the N-terminal noncatalytic domain of MEG2 (a 20-amino acid peptide corresponding to residues 63–82). The specificity of this antibody (referred to as MEG2 63–82) was confirmed using the same techniques described for the MEG2-whole molecule antibody (not shown).

The specific tyrosine phosphatase activities observed in MEG2 protein were 6.85 ± 0.82 pmol of phosphate released/10^6 cells/mg antibody for the MEG2 whole molecule antibody and 11.63 ± 0.82 pmol of phosphate released/10^6 cells/μg antibody for the MEG2 63–82 antibody (n = 3).

**MEG2 Is Predominantly Cytoplasmic in Neutrophils**—To determine the subcellular localization of MEG2, immunofluorescence localization was initially conducted in U937 cells transfected with HA-MEG2 using an anti-HA antibody. Fig. 3a illustrates that the tagged recombinant MEG2 protein is predominantly cytoplasmic. The localization of the endogenous MEG2 protein was then examined in freshly isolated neutrophils using polyclonal anti-MEG2 antibodies. These studies demonstrated that, although MEG2 was predominantly cytoplasmic, the staining was distinctly punctate or granular, suggesting that MEG2 may be localized, at least in part, to the granules of mature neutrophils. To investigate this possibility, neutrophil subcellular fractions were isolated by Percoll gradient centrifugation and analyzed by immunoblotting (see "Ex-
Experimental Procedures. These studies (Fig. 4) demonstrated that, although MEG2 is predominantly located in the cytosol, significant amounts are present in the secondary granules and a fraction containing secretory vesicles and plasma membrane. At longer exposures, minor amounts of immunoreactive MEG2 were also observed in the tertiary granule fraction (not shown). Because the immunofluorescence studies (Fig. 3) did not demonstrate a staining pattern consistent with plasma membrane localization, we concluded that the signal from the combined plasma membrane/secretory vesicle fraction was predominantly from the secretory vesicle compartment. The 68-kDa immunoreactive bands in each of these fractions could be competed in the presence of the immunizing protein (not shown), confirming the specificity of detection.

MEG2 Phosphatase Activity Is Inhibited by Agonist Stimulation—Activation of leukocytes results in increased levels of cellular tyrosine phosphorylation that parallels inhibition of tyrosine phosphatases such as SHP-1 (17) and CD45 (20). To determine if the catalytic activity of MEG2 was modulated by agonist exposure, phosphatase activity was quantified in MEG2 immunoprecipitates (using MEG2 62–83 antibody) from quiescent and activated neutrophils. Fig. 5 illustrates that the enzymatic activity of MEG2 was inhibited following treatment with either phorbol myristate acetate (PMA) or the phagocytic stimulus, opsonized zymosan. Treatment of neutrophils with diamide, a potent oxidizing agent and known phosphatase inhibitor (38, 39), resulted in the greatest degree of inhibition of MEG2 activity. However, inhibition of MEG2 was not universally associated with agonist exposure, because stimulation of neutrophils with the chemoattractant fMLP, which binds to a receptor of the seven-transmembrane-spanning domain family, did not result in inhibition of...
MEG2. These observations suggest that the activity of MEG2 is selectively modulated during neutrophil stimulation and may regulate tyrosine phosphorylation-dependent signaling events involved in microbicidal processes such as phagocytosis.

The activity of other tyrosine phosphatases such as SHP-1 is regulated by phosphorylation on serine/threonine (17, 40) or tyrosine (41-43) residues. To investigate the potential role of phosphorylation in regulation of the enzymatic activity of MEG2, its phosphorylation status was assessed in quiescent and stimulated cells. Two methods to assess phosphorylation in anti-MEG2 immunoprecipitates were used. First, cells were labeled with [32P]orthophosphate followed by immunoprecipitation of MEG2, SDS-PAGE, and autoradiography. Second, MEG2 immunoprecipitates were immunoblotted with the phosphotyrosine-specific antibody 4G10. However, neither method detected phosphorylation of MEG2 in either resting or stimulated cells (not illustrated).

**Mechanism of MEG2 Regulation: Studies with Recombinant MEG2**—To study factors regulating MEG2 enzymatic activity, a series of GST fusion protein constructs, including full-length wild type MEG2, mutant C515S MEG2, the C-terminal catalytic domain, and the N-terminal CRALBP noncatalytic domain were engineered and expressed in bacteria. The function of this domain is not known but, by analogy, may be involved in lipid metabolism, transport, or binding. We hypothesized that the noncatalytic domain regulates the catalytic activity of the phosphatase domain, as has been reported for SHP-1 (44, 45) and SHP-2 (46, 47). To investigate this possibility, we engineered separate GST constructs of the catalytic and the N-terminal domains. As illustrated in Fig. 6, the specific activity of the isolated catalytic domain (MEG2-Cat) was approximately double the activity of the full-length MEG2 protein on an equimolar basis. This observation suggests that the N-terminal retinaldehyde binding protein (CRALBP) domain of MEG2 exerts an inhibitory effect on the holoenzyme. To explore this possibility further, we repeated the assay of activity of the catalytic domain in the presence of equimolar amounts of the N-terminal domain. However, contrary to our predictions, no inhibitory effect was observed (not shown).

**MEG2 Is Activated by Polyphosphoinositides**—Because the N-terminal domain of MEG2 has homology to lipid-binding proteins, we postulated that the activity of the phosphatase might be regulated by presence of lipids. Accordingly, we studied the effects of various lipids on the activity of the MEG2 fusion proteins (Fig. 7). In our initial studies, lipids were bound to octyl-Sepharose beads and then incubated with recombinant MEG2. As illustrated by Fig. 7a, the activity of MEG2 was increased more than 3-fold by PI 4,5-P2 compared with no other test lipids were present in minor quantities (5% molar).

![Fig. 5. Agonist-induced modulation of MEG2 activity.](image)

**Fig. 5.** Agonist-induced modulation of MEG2 activity. Neutrophil suspensions were treated without (control) or with the indicated agents for 10 min, except fMLP stimulation, which was for 2 min. a, tyrosine phosphatase activity of MEG2 determined in vitro following immunoprecipitation (see "Experimental Procedures"). Results (mean ± S.D.) of four experiments are presented with the background corrected for nonimmune IgG control. Asterisks indicate statistical significance versus unstimulated control. b, immunoblotting of the immunoprecipitates confirmed that equivalent amounts of MEG2 were immunoprecipitated following stimulation with each agent.

![Fig. 6. GST-MEG2 fusion protein studies: in vitro phosphatase activity.](image)

**Fig. 6.** GST-MEG2 fusion protein studies: in vitro phosphatase activity. Equal amounts of fusion protein (0.1 µg) were incubated in an assay buffer containing p-nitrophenyl phosphate. Samples were incubated for 30 min shaking at 37°C and then measured as described under "Experimental Procedures." Values represent mean ± S.D. of n = 4 experiments. The asterisk indicates statistical significance as compared with bovine serum albumin control.
lipin, did not affect MEG2 activity demonstrating that the effect of phosphatidylinositol phosphates on MEG2 is not simply based on charge interactions.

To test the positional specificity of MEG2 activation by PI 4,5-P₂, phosphatidylcholine liposomes were prepared containing positional isomers of phosphatidylinositol phosphate (Fig. 7c). In addition to PI 4,5-P₂ and PI 4-P, significant activation of MEG2 phosphatase activity was observed with PI 3,4,5-P₃. Other isomers tested had only minor and statistically insignificant effects on the activity of MEG2. Finally, PI 4-P, PI 4,5-P₂, and PI 3,4,5-P₃, when presented in mixed phosphatidylcholine liposomes, had no significant effect on the activity of the related PTP SHP-1 (not shown), indicating the specificity of the phospholipid activation on MEG2.

MEG2 Associates with Phagosomes—Neutrophil microbicidal activity is dependent on internalization (phagocytosis) of microorganisms followed by fusion of cytoplasmic granules with the phagosome and release of a variety of cytotoxic compounds that degrade the ingested organism. The observations that MEG2 activity is modulated during phagocytosis of opsonized zymosan and that the phosphatase is associated with granules known to fuse with nascent phagosomes, suggested that MEG2 might be associated with phagosomes. To investigate this possibility, phagosomes were isolated from neutrophils using serum-opsonized magnetic beads. Fig. 8a illustrates a time-dependent increase in the amount of MEG2 associated with the maturing phagosome. Minimal binding of MEG2 to the opsonized beads was observed if phagocytosis was prevented by pretreatment of cells with either latrunculin B, a cytoskeletal disrupting agent, or the PI3K inhibitor wortmannin, both known inhibitors of phagocytosis (Fig. 8b). Other phagosomal markers copurified with MEG2 in the phagosome preparations including LAMP2, and gp91phox (Fig. 8, c and d). These observations also confirm the validity of the phagosomal preparations.

**DISCUSSION**

In the present study we have demonstrated that the tyrosine phosphatase MEG2 is expressed by human peripheral blood neutrophils and cultured cell lines of myeloid origin. The initial
pretreated for 1 h with serum-opsonized iron oxide beads. For control purposes and to inhibit phagocytosis, neutrophils were allowed to phagocytose for the indicated times. Cells were lysed, and the proteins were separated by SDS-PAGE and probed for the indicated proteins using Western blotting.

Specifically, in concert with the homology of the N-terminal domain of MEG2 with yeast Sec14P and CRALBP, this granular/vesicular association suggests the possibility that MEG2 functions in signaling or membrane fusion events involved in phagocytosis and/or maturation of the phagosome such as endosomal-phagosomal fusion (37, 48). Our observation that the enzymatic activity of MEG2 is modulated during phagocytosis is also consistent with a role for the phosphatase in the signaling events associated with phagocytosis.

It has recently been reported that a recombinant MEG2 fusion protein, heterologously expressed in Jurkat cells, localized to large, round cytoplasmic organelles of uncertain origin (23). We have observed a similar pattern of immunolocalization of endogenous MEG2 in Jurkat cells and primary CD4+ T-lymphocytes using the anti-MEG2 antibodies described in this report. These observations highlight differences in the subcellular localization (and potentially function) of MEG2 in lymphoid and myeloid cells. The precise localization of MEG2 in lymphoid cells and the significance of these “cytoplasmic organelles” in lymphoid cells remain unclear and require additional investigation.

The noncatalytic (N-terminal) domain of MEG2 shares significant homology with the proteins CRALBP and yeast SEC14P. Because both of these proteins are known to bind hydrophobic ligands, it has been suggested that the function of MEG2 may also involve the binding of lipids (21). Indeed, in the current manuscript, we have provided definitive evidence that the activity of MEG2 is stimulated by polyphosphoinositides. Specifically, using two distinct assay systems, PI 4,5-P₂ consistently enhanced the activity of MEG2 by 5- to 8-fold. We also observed that the structurally related phospholipids, PI 3,4,5-P₃ and PI 3,4,5-P₄, and, to a lesser extent, PI 4-P, also stimulate MEG2 activity. However, this activation is not simply due to the anionic nature of PI 4,5-P₂, because another highly anionic phospholipid, cardiolipin, failed to activate MEG2. We speculate that MEG2 associates with phospholipids present in the membranes of granular or vesicular compartments via its noncatalytic N-terminal domain leading to spatially restricted activation. Future studies will determine the significance of MEG2-lipid interactions in vivo and elucidate which polyphosphoinositides are of physiological relevance.

A functional role for the noncatalytic domain of MEG2 is further suggested by the studies that compared the activity of the full-length GST-MEG2 construct with that of the C-terminal phosphatase domain. These studies revealed that the catalytic activity of the single-domain construct was significantly enhanced when compared with full-length MEG2 suggesting that the noncatalytic domain inhibits the activity of the phosphatase domain. Based on these observations, we propose a model for the functional regulation of MEG2. In the resting state, MEG2 assumes a conformation in which interactions between the two domains restrain the catalytic potential of the phosphatase domain. Interaction of the N-terminal domain with membrane phospholipids such as PI 4,5-P₂ or PI 3,4,5-P₃ or other binding partners releases its autoinhibitory function resulting in enhanced catalytic activity. It should be recalled that, in experiments where the N-terminal domain fusion was mixed in equimolar amounts with the C-terminal catalytic domain fusion, no inhibition of the C-terminal domain catalytic activity was observed. This may reflect the inability to reconstruct complex intermolecular interactions with separate fusion proteins in vitro in dilute solution. We also investigated whether inter-domain interactions required the presence of lipids, but no effect of the N-terminal domain was observed on the catalytic activity of the C-terminal domain after the addi-
tion of a variety of lipids. It is therefore possible that the conformations assumed by our fusion proteins do not sufficiently resemble the physical states of the domains within the native molecule that are required for inhibitory interactions to occur. Further studies are required to explore this possibility.

The role of specific protein-tyrosine phosphatases in myeloid leukocyte function is beginning to be defined. CD45 for example has been demonstrated to be critical for the maintenance of integrin-mediated adhesion in bone marrow-derived macrophages (49). Activation of the Src family members p65(ck) and p59(Fyn) is dependent on a CD45-mediated dephosphorylation of these kinases (50). Overexpression of SHP-1 in established myeloid cell lines has been shown to suppress cell growth (51), and SHP-1 has been shown to associate with and regulate the function of a number of growth factor receptors, including the epidermal growth factor, platelet-derived growth factor, Kit, and interleukin-3 receptors (52). Recent studies from our laboratory have shown that SHP-1 negatively regulates adhesion and the oxidative burst in myeloid cells in response to PMA stimulation (53). In light of our evidence presented here that MEG2 associates with nascent phagosomes, is catalytically regulated during phagocytosis, is activated by PI 4,5-P2 or PI 3,4,5-P3, we speculate that MEG2 participates in regulation of signaling or membrane fusion events involved in phagocytosis. These observations have potentially important implications for our understanding of the molecular mechanisms regulating phagocytosis, an essential antimicrobial function of neutrophils.

MEG2, a Polyphosphoinositide-activated Tyrosine Phosphatase

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Protein-tyrosine Phosphatase MEG2 Is Expressed by Human Neutrophils: LOCALIZATION TO THE PHAGOSOME AND ACTIVATION BY POLYPHOSPHOINOSITIDES

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