Presqualene diphosphate (PSDP) is a bioactive lipid that rapidly remodels to presqualene monophosphate (PSMP) upon cell activation (Levy, B. D., Petasis, N. A., and Serhan, C. N. (1997) Nature 389, 985–990). Here, we have identified and characterized a phosphatase that converts PSDP to PSMP. Unlike the related polyisoprenyl phosphate farnesyl diphosphate (FDP), PSDP was not a substrate for type 2 lipid phosphate phosphohydrolases. PSDP phosphatase activity was identified in activated human neutrophil (PMN) extracts and partially purified in the presence of Nonidet P-40 with gel filtration and anion exchange chromatography. Peptide sequencing of a candidate phosphatase was consistent with polyisoprenic acid phosphatase domain containing 2 (PPAPDC2), an uncharacterized protein that contains a lipid phosphate phosphohydrolase consensus motif. Recombinant PPAPDC2 displayed diphosphate phosphatase activity with a substrate preference for PSDP > FDP > phosphatidic acid. PPAPDC2 activity was independent of Mg²⁺ and optimal at pH 7.0 to 8.0. Incubation of [¹⁴C]FDP with recombinant human squalene synthase led to [¹⁴C]PSDP and [¹⁴C]squalene formation, and in the presence of PPAPDC2, [¹⁴C]PSMP was generated from [¹⁴C]PSDP. PPAPDC2 mRNA was detected in human PMN, and is widely expressed in human tissues. Together, these findings indicate that PPAPDC2 in human PMN is the first lipid phosphate phosphohydrolase identified for PSDP. Regulation of this activity of the enzyme may have important roles for PMN activation in innate immunity.

Neutrophil (PMN) activation plays a central role in diverse responses such as host defense, inflammation, and reperfusion injury (1). In response to inflammatory stimuli, PMN target reactive oxygen species and granule enzymes to phagolysosomes for microbial killing or digestion of foreign materials (2). Anomalous release of these potentially toxic agents often occurs, amplifying inflammation and leading to tissue injury, events that are implicated in a wide range of human diseases (3). To prevent an excessive inflammatory response and limit damage to the host, PMN pro-inflammatory and anti-inflammatory signals (4).

It is now well appreciated that several enzymes of lipid metabolism also serve as signaling modules with their products acting as bioeffectors (5, 6). Polyisoprenyl phosphates, specifically the mevalonate-derived product presqualene diphosphate (PSDP), carry biological activity as an intracellular down-regulatory signal in human PMNs (7). PSDP directly inhibits phospholipase D (PLD) and leukocyte superoxide anion generation in vitro and in vivo (8, 9). Recently, the hyperimmunoglobulinemia D and periodic fever syndrome were identified as a systemic inflammatory illness stemming from partial deletion of mevalonate kinase and subsequently decreased isoprenoid production (10, 11). Together, these observations indicate that in addition to their roles as structural elements in cholesterol biosynthesis, mevalonate-derived products can also display properties of lipid mediators in inflammation.

Cell activation by receptor-mediated agonists leads to rapid and transient polyisoprenyl phosphate remodeling (7, 8). PSDP is present in freshly isolated human PMN. When cells are activated, PSDP shifts from perinuclear to granule and microsomal subcellular domains, and within seconds is converted to its monophosphate form, presqualene monophosphate (PSMP) (7, 12). As an inhibitor of PLD and reactive oxygen species generation, PSMP is over 100-fold less potent than PSDP (8). These findings suggest the presence of a regulated diphosphate phosphatase. Here, we report the identification of the first PSDP phosphatase in human PMN and characterize its biochemical properties.

**EXPERIMENTAL PROCEDURES**

**Materials**—Phosphatidic acid (PA, C10:0) and diacylglycerol pyrophosphate (DGPP, C18:1) were purchased from Avanti Polar Lipids (Alabaster, AL) and FDP from Sigma. Leukotriene B₄ was from Cayman Chemical (Ann Arbor, MI). Plasmids encoding the LPPRP2 gene were purchased from Origene (Rockville, MD). Recombinant human phosphatidic acid phosphohydrolase 2a (PAP2a), PAP2b, and PAP2c were generated as described in Ref. 13. [¹⁴C]FDP (55 mCi/mmol, 50 μCi/ml) was from American Radiolabeled Chemicals (St. Louis, MO). PSDP was isolated from healthy human PMNs as described (7).

**Purification of PSDP Phosphatase Activity from PMN**—Human PMN were isolated from whole blood as described (7), suspended (10⁷ PMN/ml) in Hanks’ balanced salt solution with 1.6 mM CaCl₂, warmed (5 min, 37 °C), and then activated with leukotriene B₄ (100 nM, 60 s, 37 °C). Cells were then separated into two fractions by centrifugation (1,000 × g, 10 min, 4 °C). Cells and supernatants were subjected to gel filtration with Sephacryl S200–HR (Amersham Biosciences) equilibrated with Buffer A (50 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.1% Triton X-100, 1 mM PMSF, 1 mM benzamidine, 10 μg/ml aprotinin, and 10 μg/ml leupeptin).

**RESULTS AND DISCUSSION**

**Presqualene Diphosphate Phosphatase Identification and Functional Characterization.** Presqualene diphosphate (PSDP) is a bioactive lipid that rapidly remodels to presqualene monophosphate (PSMP) upon cell activation. Presqualene diphosphate phosphatase activity was identified in activated human neutrophil (PMN) extracts and partially purified in the presence of Nonidet P-40 with gel filtration and anion exchange chromatography. Peptide sequencing of a candidate phosphatase was consistent with polyisoprenic acid phosphatase domain containing 2 (PPAPDC2), an uncharacterized protein that contains a lipid phosphate phosphohydrolase consensus motif. Recombinant PPAPDC2 displayed diphosphate phosphatase activity with a substrate preference for PSDP > FDP > phosphatidic acid. PPAPDC2 activity was independent of Mg²⁺ and optimal at pH 7.0 to 8.0. Incubation of [¹⁴C]FDP with recombinant human squalene synthase led to [¹⁴C]PSDP and [¹⁴C]squalene formation, and in the presence of PPAPDC2, [¹⁴C]PSMP was generated from [¹⁴C]PSDP. PPAPDC2 mRNA was detected in human PMN, and is widely expressed in human tissues. Together, these findings indicate that PPAPDC2 in human PMN is the first lipid phosphate phosphohydrolase identified for PSDP. Regulation of this activity of the enzyme may have important roles for PMN activation in innate immunity.

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**Identification and Functional Characterization of a Presqualene Diphosphate Phosphatase.**

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Identification of PSDP Phosphatase

Expression of Recombinant PPAPDC2-His, in S21 Cells—The human PPAPDC2 cDNA was generated by RT-PCR from human PMN, inserted into the EcoRI site of pcP4-TOPO (Invitrogen, Palo Alto, CA) and verified by direct sequencing. A DNA fragment containing the coding sequence of the PPAPDC2 gene flanked by BamHI and XhoI was obtained by PCR (forward primer, 5′-AGTCGCCGATCCCGGAG-GAGCATGGAG-3′; reverse primer, 5′-CTCGAGTCGCTGAGTGGT-GGTATGATGTCAGGTCCACGAGT-3′) with Pfu polymerase using a cDNA plasmid encoding PPAPDC2 as a template. To add an additional His6 sequence at the carboxyl terminus of PPAPDC2, the reverse primer contained a 21-mer artificial sequence that codes His6 and a stop codon (indicated as underlined). The PCR product was digested with BamHI and XhoI (New England Biolabs, Inc., Beverly, MA), ligated into the same restriction enzyme sites of pBacPAK9 baculovirus vector, and expressed in insect cells using the BacPAK® Baculovirus Expression System (Clontech, San Jose, CA). S21 cells were infected with a viral multiplicity of 5 and harvested after 72 h. Cells were suspended in 50 mM HEPES (pH 7.4), 80 mM KCl, 1 mM dithiothreitol, 3 mM EDTA (100 μl total volume). Materials were incubated for 30 min at 37 °C. Each reaction was stopped with 200 μl of malachite green reagent and incubated an additional 20 min (room temperature). Absorbance at 630 nm was measured with a 96-well microplate reader (μQuant, Bio−Tek Instruments, Inc., Winooski, VT).

Preparation of Recombinant Human Squalene Synthase—Human squalene synthase (SQS) cDNA was cloned by RT-PCR from human peripheral blood leukocytes using specific primers: 5′-AGAATCTCAATGGAGT-TGCTGAAA-3′ and 5′-TATGCTGACCTGTTCTCCAGT-3′ designed according to the GenBank accession number NM_004462. The truncated soluble form of human SQS was designed as described (14) using primers: 5′-TAAATTCTAGGACAGAGCCTCTGCTCCG-3′ and 5′-TCTCTG-AAGATTCTCAGGGTTGTCG-3′. The PCR fragment was subcloned into pET21a (Novagen, Nottingham, UK), and the insert was sequenced to verify the structure. Recombinant hSQS was isolated from 1 ml isopropl 1-thio-D-galactopyranoside-induced Escherichia coli (BL21 Star, Invitrogen) containing plasmid pET21a-hSQS by freezing and thawing cell pellets in binding buffer (NaHPO4 20 mM (pH 7.8), NaCl 500 mM, imidazole 5 mM). After sonication and centrifugation (20,000 × g, 20 min), supernatants were loaded onto a nickel-nitrilotriacetic acid-agarose column (Invitrogen). After equilibration with wash buffer (20 mM NaHPO4, pH 6.0, 500 mM NaCl), His-tagged protein was eluted with stepwise increases in imidazole concentration (50, 200, 350, and 500 mM) in wash buffer. Recombinant hSQSs eluted at 350 mM imidazole was diazylated overnight in phosphatase-buffered saline, 5 mM dithiothreitol, 2 mM MgCl2, and stored in the presence of 50% glycerol at −80 °C.

Squalene Synthesis Assay—Squalene synthesis activity was determined in 100 mM potassium phosphate, 5 mM MgCl2, 5 mM CHAPS, 10 mM dithiothreitol, [14C]FDP (0.25 μCi) in 23 μM FDP and 2 μg of rhSQS protein in a total reaction volume of 10 μl. Some reactions were performed in the presence of 2 mM NADPH to promote PSDP conversion to squalene. For determination of polyprenyl phosphate phosphatase activity, PAP2a (5 μg) or PPAPDC2 (2 μg) were co-incubated with

10% glycerol, and 0.2% Nonidet P-40). The column (C10/40 (Amersham Biosciences), internal diameter 10 mm, length 40 cm) was eluted with 150 ml of Buffer A at 0.6 ml/min, and 3-ml fractions were collected. Materials eluting from the column were monitored by absorbance at 280 nm and screened for the ability to release phosphate from FDP. Eluted fractions containing phosphatase activity were combined.

Enzymatically active materials in these fractions were concentrated by spin filtration with a 10-kDa limiting membrane (Centricon YM-10, Amicon Bioseparations, Millipore, Bedford, MA), and applied to DEAE-Sepharose (DFF100, Sigma) for anion-exchange chromatography. The column (MT20 (Bio-Rad), internal diameter 15 mm, length 11.3 cm) was eluted at 1.0 ml/min for 10 ml with Buffer A and then an additional 40 ml with a linear KCl gradient (0–1.0 M) was constructed in Buffer A, and 1-ml fractions were collected. Eluents were screened for polyprenyl phosphate phosphatase activity. Fractions with PSDP phosphatase activity were combined, concentrated, and electrophoresed by SDS-PAGE with 12% polyacrylamide gel. The gel was stained for protein with Coomassie Brilliant Blue (Bio-Rad), and protein in the expected range was excised. After proteolytic digestion, sequence analysis was performed at the Harvard Microchemistry Facility by microcapillary reverse-phase high performance liquid chromatography nano-electrospray tandem mass spectrometry on a Finnigan LCQ quadrupole ion trap mass spectrometer.

Phosphatase Activity with Type 2 Lipid Phosphate Phosphatases—S21 cells were infected with baculovirus vectors containing recombinant PAP2a, PAP2b, and PAP2c at a viral multiplicity of 10 and harvested after 48 h as described in Ref. 13. Cells were suspended in 50 mM HEPES (pH 7.4), 80 mM KCl, 1 mM dithiothreitol, 3 mM EDTA (100 μl total volume). Materials were incubated for 30 min at 37 °C. Each reaction was stopped with 200 μl of malachite green reagent and incubated an additional 20 min (room temperature). Absorbance at 630 nm was measured with a 96-well microplate reader (μQuant, Bio−Tek Instruments, Inc., Winooski, VT).

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rhSQS (2 μg) in reaction buffer. Enzyme and substrate were incubated for up to 2 h at 37 °C, after which the reactions were stopped by the addition of 10 μl of 1 M EDTA (pH 9.2) (15). Each reaction mixture (10 μl) was analyzed by thin layer chromatography. Silica Gel TLC plates (Fisher) were developed with chloroform/methanol/water (65:25:4, v/v/v). The radioactivity of each spot was visualized and quantified with a Bio-imaging analyzer (model 425E; Amersham Biosciences, with ImageQuant software version 3.2). After imaging, densitometry was performed using Scion Image software.

RNA Dot Blot Hybridization of PPAPDC2 RNA—Hybridization of a 600-bp fragment encoding the open reading frame of PPAPDC2 to a human RNA multiple tissue expression array (MTE array, Clontech) was performed following the manufacturer’s instructions. Primers used to generate the human PPAPDC2 probe were 5’-CCCTTAGAAGCT-GCTGGAG-3’ (forward primer) and 5’-GGCTGCTGGTTAGGTT-GAC-3’ (reverse primer). The identity of the probe was confirmed by direct sequencing.

Statistical Analysis—Data were provided as the mean ± S.E. Comparisons of results were conducted by two-way analysis of variance followed by post-hoc Scheffe’s test. p < 0.05 was considered significant.

RESULTS

Activated PMNs display polyisoprenyl phosphate phosphatase activity by converting PSDP to PSMP (Fig. 1A), so freshly isolated human PMNs were exposed to leukotriene B4 (100 nM, 60s, 37 °C), lysed by sonication, and nuclei and remaining intact cells were pelleted. Supernatants were applied to Sephacryl S200-HR for gel filtration and 3-ml eluents were collected and assayed for polyisoprenyl phosphate phosphatase activity using FDP (see “Experimental Procedures”). We chose to screen the column fractions with FDP, because it is closely related to the structure of PSDP and quantities of PSDP were more limited. FDP phosphatase activity eluted with proteins of an estimated molecular mass of ~35 kDa after staining with Coomassie Brilliant Blue. E, recombinant hPAP2a (5 μg) was incubated (30 min, 37 °C) with PSDP, PA (C10:0), FDP or DGPP (10 μM), and phosphatase activity was determined by phosphate release (see “Experimental Procedures”). Values are mean ± S.E., n = 3, *, p < 0.01 compared with vehicle.
the catalytically active column fractions after gel filtration, concentrated the fractions by spin filtration with a 10-kDa limiting membrane, and then applied the materials to DEAE-Sepharose for anion-exchange chromatography (see “Experimental Procedures”). The column eluents were again screened for polyisoprenyl phosphate phosphatase activity with FDP and a broad peak of activity was identified over 5 separate 1-ml column fractions (Fig. 1B). For each fraction, we next compared FDP to PSDP phosphatase activity and determined that fractions from the leading edge (fractions 27 and 28) had significantly greater phosphatase activity for FDP than PSDP when assayed in parallel (Fig. 1C). In sharp contrast, fractions 31 and 32 from the trailing edge of the peak displayed a preference for PSDP as a substrate. The materials in fractions 27 and 28 and fractions 31 and 32 were combined, separated by 12% SDS-PAGE, and Coomassie Blue staining revealed a prominent band of protein at $\sim 35$ kDa (Fig. 1D). Type 2 phosphatidic acid phosphohydrolase (PAP2) isoforms are $\sim 35$ kDa, involved in lipid signaling, and can dephosphorylate FDP (16). To determine whether isoforms of PAP2 also displayed PSDP phosphatase activity, we first expressed human PAP2a and incubated recombinant protein (30 min, 37 °C) with PSDP, PA (C10:0), FDP, and DGPP (C18:1) (1.91 ± 0.45, 2.72 ± 0.7, and 3.17 ± 0.11 nmol/min/mg, respectively, Fig. 1E). In sharp contrast, phosphate release from PSDP was significantly less efficient (0.20 ± 0.03 nmol/min/mg, $p < 0.01$). In separate experiments, rhPAP2b and rhPAP2c were also expressed and similar to PAP2a, these isoforms did not have significant phosphatase activity for PSDP (data not shown). These results indicated that PSDP, unlike shorter isoprenoid diphosphates, was not a substrate for type 2 lipid phosphate phosphohydrolases.

To identify the candidate PSDP phosphatase, enzymatically active materials for PSDP were extracted from the SDS-PAGE gel (Fig. 1D). After proteolytic digestion, peptide sequencing uncovered a partial amino acid sequence, AGPSQSXXXXXXE, that was found in phos-
Identification of PSDP Phosphatase

Phosphatidic acid phosphatase type 2 domain containing 2 (PPAPDC2) (GenBank accession number BC038108), an uncharacterized but closely related protein to PAP2A (Fig. 2A). PPAPDC2 encodes a protein of 295 amino acids with an anticipated molecular mass of 31.5 kDa. The PPAPDC2 sequence displayed an overall 17% identity and 18% similarity to human PAP2A (Fig. 2A). Of note, PPAPDC2 had substantially greater homology with PAP2A in three conserved domains (C1, C2, and C3) that comprise a putative lipid phosphate phosphatase sequence motif, K(X)₉RP-(X₂₋₅)-SGH-(X₁₀₋₁₅)-SR(X)(H)(X)₃D (Fig. 2, A and B). This motif is shared among several yeast and mammalian lipid phosphate phosphatases (17, 18). Kyte-Doolittle analysis of the overall hydropathy profile of the protein predicted a structure for PPAPDC2 similar to PAP2a with six membrane-spanning domains in the encoded protein (data not shown). Because these structural features suggested that PPAPDC2 had the ability to serve as a new member of this class of enzymes, we next determined its relationship by evolutionary hierarchy to PAP2a and other proteins with elements of the conserved PAP2 phosphatase motif (Fig. 2C). The resulting phylogenetic tree suggested that PPAPDC2, like Dolpp1 (19), would have enzymatic properties distinct from PAP2 isoforms. Investigation of cellular extracts from human PMN and HEK 293 cells indicated that these diverse cell types both carried PSDP phosphatase activity, so we next determined which of the lipid phosphate phosphatases in Fig. 2C were expressed in both cell types. In addition to PAP2a, mRNA for only PPAPDC2 and LPPRP2 was detectable by RT-PCR in both cell types (Fig. 2D). Together, these findings indicated that PPAPDC2 was a candidate PSDP phosphatase.

To investigate whether PPAPDC2 or LPPRP2 had PSDP phosphohydrolase activity, S21 cells were transfected with baculovirus vectors for expression of recombinant protein. In membrane fractions with rhPPAPDC2, a band with an apparent molecular mass of 31.5 kDa was specifically detected, and the enzyme was partially purified (>90%) with a Ni²⁺-charged column (Fig. 3A). Recombinant LPPRP2 was similarly expressed and purified (data not shown). PSDP phosphatase activity for PPAPDC2 was dependent on substrate concentration (Fig. 3B). No PSDP phosphatase activity was present with rLPPRP2 (data not shown).

PPAPDC2 demonstrated a marked substrate preference for PSDP over PA (C10:0) (Fig. 3, B and C). In these reactions, PPAPDC2 exhibited -5-fold more phosphate release from PSDP than equimolar amounts (10 μM) of the other substrates tested with a rank order of PSDP > FDP > PA > DGPP (Fig. 3C). This structure-activity relationship for PPAPDC2 is in sharp contrast to that observed with rhPAP2a (Fig. 1E).

We next examined the effect of pH, divalent cations, and detergent on PPAPDC2 phosphatase activity. The impact of pH on PSDP phosphatase activity was determined at equimolar concentrations of PSDP (10 μM) (Fig. 4A). PPAPDC2 exhibited a pH optimum between 7.0 and 8.0, so activity was routinely measured at pH 7.4. We also determined the impact of divalent cations on PPAPDC2 activity. PSDP phosphatase activity was independent of Mg²⁺, Mn²⁺, Ca²⁺, and Zn²⁺ concentrations between 1 and 10 mM (data not shown). When PSDP phosphatase activity was determined with PPAPDC2 in the presence of 0.1–1% Triton X-100/phospholipid-mixed micelles, the activity was maximal at 0.1% Triton X-100 (data not shown). Experiments using other detergents, including Nonidet P-40 and β-octyl glucoside, at concentrations of 0.1–1% were also performed, but none proved superior to 0.1% Triton X-100.

Squalene synthase catalyzes the head-to-head condensation of two molecules of FDP to generate PSDP as a biosynthetic intermediate in the two-step conversion of FDP to squalene (20). To determine whether PPAPDC2 converted PSDP to PSMP and/or its primary alcohol, presqualene alcohol, we utilized [¹⁴C]PSDP that was generated in vitro by rhSQS and tracked the metabolic fate of FDP and PSDP by phosphorimaging after thin layer chromatography. Incubation of [¹⁴C]FDP with rhSQS (2 μg) and NADPH (2 mM) led to [¹⁴C]PSDP and [¹⁴C]squalene biosynthesis. In the presence of rhPPAPDC2 (2 μg), [¹⁴C]PSDP was converted to both [¹⁴C]PSMP (3.1 nmol of PSMP/min/mg) and [¹⁴C]squalene (Fig. 4, B and 4C) with 15.4 ± 1.2% conversion of PSDP to PSMP and 46.9 ± 5.1% of PSDP conversion to squalene (n = 4, Fig. 4B). No presqualene alcohol was detected up to 120 min. The time-dependent conversion of PSDP to PSMP gave a two-parameter hyperbolic relationship (y = 1.5(x))/116.8 + x, r² = 0.998, p < 0.0001) (Fig. 4C). In addition, PPAPDC2 also catalyzed FDP dephosphorylation to FMP (10.1 ± 1.9%, n = 4, Fig. 4B), but displayed a significantly increased preference for PSDP as a substrate (p < 0.05, n = 4). No farnesol was detected. Together, these in vitro results indicated that rhPPAPDC2 was a polysoprenyl phosphate diphosphate phosphatase and that, in vitro, PSDP generated by rhSQS was also available, despite its intimate relationship with this enzyme, for interactions with other proteins and conversion to products (i.e. PSMP) other than squalene.
To determine the impact of PPAPDC2 and PAP2a on polyisoprenyl phosphate remodeling, co-incubations of the enzymes were performed with rhSQS in the presence of [14C]FDP. Recombinant hSQS generated PSDP (Fig. 5A, lane 2), but required NADPH for conversion to squalene with 5 and 2% conversion of FDP to PSDP and squalene, respectively, after 120 min (37 °C) (Fig. 5A, lane 3). In these incubations, rhSQS converted 40% of PSDP to squalene. PSMP formation was dependent on the presence of rhPPAPDC2 and independent of NADPH (lanes 4 and 5). When both rhSQS and PPAPDC2 were present (lane 5), PSDP formation increased (9.8 to 17.7% conversion from FDP). Of note, the rate of squalene generation from PSDP (38.5%) was without significant change from incubations with rhSQS alone (36.9%) (lane 3). Neither boiled rhPPAPDC2 (lane 6) nor intact rhPAP2a (lane 7) converted PSDP to PSMP. Co-incubation of rhPAP2a and rhSQS markedly enhanced formation of FMP from FDP (24.6%) and in addition, rhPAP2a dephosphorylated FMP to generate farnesol (lanes 7 and 8). Because the amounts of FDP present in these incubations exceeded the rate-limiting concentrations for both rhSQS and rhPAP2a, their co-incubation did not significantly affect the rates of PSDP or squalene synthesis (lane 7). Although PSDP was generated by rhSQS, no PSMP was detected in the presence of PAP2a. In the absence of rhSQS, PAP2a converted FDP to FMP (18.3%) and farnesol (5.0%) without formation of PSDP, PSMP, or squalene (lane 8). Together, these findings confirmed that PPAPDC2 was a polyisoprenyl phosphate diphosphate phosphatase (Fig. 5B), and that PAP2a did not utilize PSDP as a substrate. In addition, PAP2a also differs in its biochemical properties from PPAPDC2 in its ability to dephosphorylate both polyisoprenyl diphosphates and monophosphates.

To determine the pattern of expression for PPAPDC2 mRNA, we performed RNA blot hybridization with a human tissue RNA expression array. In addition to human PMN and HEK 293 cells, PPAPDC2 was also expressed in most organs, in particular gastrointestinal organs, spleen, placenta, kidney, thymus, and brain (Fig. 6A). Semi-quantitative RT-PCR revealed PPAPDC2 expression in human PMN that was ~60% of levels in human liver (Fig. 6B).

DISCUSSION

Exposure of human PMN to receptor-mediated stimuli triggers the rapid conversion of PSDP to PSMP (7), indicating activation of a PSDP diphosphate phosphatase. Polyisoprenyl phosphate remodeling in PMN temporally overlaps intracellular signaling events (e.g. PLD activation) and functional responses (e.g. O2 production) (8, 12). PSDP is over 100-fold more potent than PSMP as an inhibitor of PLD and PMN NADPH oxidase assembly (7, 8), and structural mimetics of PSDP can decrease PMN trafficking and activation in vivo during acute inflammatory responses (9). Together, these findings suggest that decrements in cellular PSDP levels by a PSDP phosphatase would play a pivotal role in regulating leukocyte function. Here, we have identified PPAPDC2 as the first PSDP phosphatase from human PMN and characterized the biochemical properties of this member of the lipid phosphatase/phosphotransferase (LPT) family.
LPTs comprise a large multigene family with at least five distinct groups, namely lipid phosphate phosphatases (LPPs), sphingosine-1-phosphate phosphatases, sphingomyelin synthase, lipid phosphate-related proteins, and a group that was recently provisionally labeled as candidate sphingomyelin synthases type 2 (CSS2s) because of sequence homology with sphingomyelin synthases (21, 22).

**PPAPDC2** (GenBank accession number BC038108) shares a conserved acid phosphatase domain with LPT family members, and close inspection of its sequence revealed its identity as the newly labeled **CSS2/H9252** (SwissProt/TrEMBL accession number Q8IY26) (21). Similar to the LPPs, PPAPDC2 is predicted by hydropathy analysis to be an integral membrane protein and possess six transmembrane helices. Despite its name, this protein does not exhibit sphingomyelin synthase activity (21) and up until this report its enzymatic properties have remained incompletely characterized (21, 22).

**CSS2/H9252** (i.e. PPAPDC2) has an entirely conserved phosphatase motif and here displayed lipid phosphate phosphatase activity in vitro for more than one polyisoprenyl phosphate substrate. The biochemical properties of PPAPDC2 were also distinct from LPPs, as PPAPDC2 had a substrate preference for PSDP with a rank order of PSDP > FDP > PA > DGPP, differing markedly from PAP2a, PAP2b, and PAP2c. These LPPs did not utilize PSDP as a substrate for phosphate release and displayed phosphatase activity for an otherwise broad range of substrates. In addition, PPAPDC2 carried only polyisoprenyl diphosphate, not monophosphate, phosphatase activity, as opposed to PAP2a that demonstrated properties of both a di- and monophosphate phosphatase. The unique preference of PPAPDC2 for diphosphates also differs from the NH2 terminus of soluble epoxide hydrolase that, similar to PAP2a, carries phosphatase activity for both polyisoprenyl di- and monophosphates (23).

In addition to its enzymatic activity, **PPAPDC2 RNA and protein expression were evident in human PMN.** In freshly isolated cells, the largest quantities of PSDP are present in PMN nuclear and granule subcellular fractions (12). Upon exposure to leukotriene B4, a PMN chemoattractant and secretagogue, PSDP levels rapidly decrease in nuclear and increase in granule-enriched fractions. Of interest, a subtractive proteomic analysis suggests that PPAPDC2 (i.e. CSS2β) localizes in part to liver nuclear membranes (24). If also present in the PMN nuclear envelope, this phosphatase would be well positioned to effect the PSDP remodeling observed upon cell activation. Here, the PSDP phosphatase activity was present in PMN and HEK 293 cell 1000 g supernatants and 10,000 g pellets, indicating that PPAPDC2 is likely to also be present in microsomal and granule-enriched fractions in these cells, similar to other LPTs (22).

**PPAPDC2 displayed a wide tissue distribution.** Expression profiles obtained for CSS2β from online data of expressed sequence tag transcript abundance similarly predict a broad mammalian tissue distri-
bution for this gene (22). In addition, the relative abundance of PPAPDC2 RNA, like other LPTs (22), was highest in gastrointestinal organs, placenta, and brain. Of interest in the regulation of inflammatory responses, there was also significant PPAPDC2 expression in immune organs (namely spleen and thymus) that are critical for myeloid, dendritic cell, and B- and T-lymphocyte development, survival and trafficking. Like PPAPDC2, sphingosine-1-phosphate phosphatases are expressed in the thymus and leukocytes (22), and sphingosine 1-phosphate and its cognate receptors can comprise autocrine and paracrine signaling networks in the regulation of leukocyte responses (25).

Inflammation and metabolic pathways for sphingosine 1-phosphate and other LPT substrates have been linked to sterol metabolism (26). PMN are innate immune effectors that are uniquely unable to generate sterols from endogenous sources of mevalonate (27). These cells lack squalene epoxidase and other mixed function oxidases required for sterol biosynthesis, leading to a biosynthetic block at SQS. Because PSDP forms an enzyme-intermediate complex in the SQS catalyzed two-step conversion of FDP to squalene, we investigated whether this polyisoprenyl phosphate was available for interactions with other proteins by co-incubating rhSQS with rhPPAPDC2. Our results provide direct evidence for PSDP conversion to PSMP in these incubations. Isoprenoid regulatory proteins have been identified at several steps in cholesterol biosynthesis to regulate flux through the pathway (28, 29) or divert biosynthetic intermediates to other functions (30). Our results indicate that PSDP is available in vitro for metabolic fates other than squalene and suggest that PSDP could interact with intracellular protein targets, such as PLD and SH2 domains (31), in the regulation of PMN functional responses.

In summary, this is the first identification of a PSDP phosphatase. Identified by functional characterization and direct sequencing, PPAPDC2 (aka CSS2β) is an LPT with polyisoprenyl diphosphate phosphatase activity and is expressed in human leukocytes and multiple human tissues. Moreover, this is the first enzymatic activity identified for a member of the CSS2 group of LPTs. Based on its newly identified biochemical properties, conservation of the LPP phosphatase motif and lack of sphingomyelin synthase activity, we propose that this protein be renamed as distinct LPT (e.g. polyisoprenyl diphosphate phosphatase 1). Regulation of the activity of the enzyme is predicted to have important roles in leukocyte function during immune responses.

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REFERENCES
1. Nathan, C. (2002) Nature 420, 846–852
2. Weissmann, G., Smolen, J. E., and Korchak, H. M. (1980) N. Engl. J. Med. 303, 27–34
3. Weiss, S. J. (1989) N. Engl. J. Med. 320, 365–376
4. Serhan, C. N., and Levy, B. D. (2003) in The Neutrophil (Cassatella, M. A., ed) Vol. 83, pp. 115–145, Karger, Basel
5. Puterman, A. H., and Hannum, Y. A. (2004) EMBO Rep. 5, 777–782
6. Newman, J. W., Morisseau, C., Harris, T. R., and Hammock, B. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1558–1563
7. Levy, B. D., Petasis, N. A., and Serhan, C. N. (1997) Nature 389, 985–990
8. Levy, B. D., Fokin, V. V., Clark, J. M., Wakeland, M. J., Petasis, N. A., and Serhan, C. N. (1999) FASEB J. 13, 903–911
9. Levy, B. D., Hickey, L., Morris, A. J., Larvie, M., Keledjian, R., Petasis, N. A., Bannenberg, G., and Serhan, C. N. (2005) Br. J. Pharmacol. 146, 344–351
10. Drenth, J. P., and van der Meer, J. W. (2001) N. Engl. J. Med. 345, 1748–1757
11. Takada, K., Akentievich, I., Mahadevan, V., Dean, J. A., Kelley, R. L., and Kastner, D. L. (2003) Arthritis Rheum. 48, 2645–2651
12. Levy, B. D., and Serhan, C. N. (2000) Biochem. Biophys. Res. Commun. 275, 739–745
13. Roberts, R., Sciorra, V. A., and Morris, A. J. (1998) J. Biol. Chem. 273, 22059–22067
14. Thompson, J. F., Danley, D. E., Mazzalupo, S., Milos, P. M., Lira, M. E., and Harwood, H. J., Jr. (1998) Arch. Biochem. Biophys. 350, 283–290
15. Gu, P., Ishii, Y., Spencer, T. A., and Shechter, I. (1998) J. Biol. Chem. 273, 12515–12525
16. Faulkner, A., Chen, X., Rush, J., Horazdovsky, B., Waechter, C. J., Carman, G. M., and Sternweis, P. C. (1999) J. Biol. Chem. 274, 14831–14837
17. Brindley, D. N., and Waggoner, D. W. (1998) J. Biol. Chem. 273, 24281–24284
18. Stukey, J., and Carman, G. M. (1997) Protein Sci. 6, 469–472
19. Rush, J. S., Cho, S. K., Jiang, S., Hofmann, S. L., and Waechter, C. J. (2002) J. Biol. Chem. 277, 45226–45234
20. Rilling, H. C., Poulier, C. D., Epstein, W. W., and Larsen, B. (1971) J. Am. Chem. Soc. 93, 1783–1785
21. Huitema, K., van den Dikkenberg, J., Brouwers, J. F., and Holthuis, J. C. (2004) EMBO J. 23, 33–44
22. Sigal, Y. J., McDermott, M. I., and Morris, A. J. (2005) Biochem. J. 387, 281–293
23. Tran, K. L., Aronov, P. A., Tanaka, H., Newman, J. W., Hammock, B. D., and Morisseau, C. (2005) Biochemistry 44, 12179–12187
24. Schirmer, E. C., Flores, L., Guan, T., Yates, J. R., 3rd, and Gerace, L. (2003) Science 301, 1380–1382
25. Rosen, H., and Goetzl, E. J. (2005) Nat. Rev. Immunol. 5, 560–570
26. Saha, D. J., and Hla, T. (2004) Circ. Res. 94, 724–734
27. Shechter, I., Fogelman, A. M., and Popjak, G. (1980) J. Lipid Res. 21, 277–283
28. Shihata, N., Arita, M., Minak, Y., Dohmae, N., Takio, K., Ono, T., Inoue, K., and Arai, H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 2244–2249
29. Tchen, T. T., and Bloch, K. (1957) J. Biol. Chem. 226, 921–930
30. Schmidt, R. A., Schneider, C. J., and Glomset, J. A. (1984) J. Biol. Chem. 259, 10175–10180
31. Levy, B. D., and Serhan, C. N. (2003) Cell. Mol. Life Sci. 59, 1–13