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*J Immunol* 2007; 179:8350-8356; doi: 10.4049/jimmunol.179.12.8350

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Activation of Phosphatase and Tensin Homolog on Chromosome 10 Mediates the Inhibition of FcγR Phagocytosis by Prostaglandin E2 in Alveolar Macrophages

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PGE2 has important inhibitory effects on the macrophage host defense functions of phagocytosis and killing, yet the molecular mechanisms involved remain to be fully elucidated. PGE2 causes an elevation of cAMP in alveolar macrophages (AMs), which in turn activates the cAMP effector targets, protein kinase A and the exchange protein activated by cAMP (Epac)-1. We now report that FcγR-induced PI3K/Akt and ERK-1/2 activation are inhibited by PGE2 in AMs. By specifically inhibiting the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in AMs, we attenuated the inhibitory effects of both PGE2 and a specific Epac-1 agonist (8-pCPT-2′-O-Me-cAMP) on FcγR-mediated phagocytosis and Akt/ERK-1/2 activation; PTEN inhibition also decreased PGE2-induced suppression of bacterial killing by AMs. Moreover, PGE2 and the Epac-1 agonist induced an increase in PTEN lipid phosphatase activity, and this was associated with decreased tyrosine phosphorylation on PTEN—a mechanism known to regulate PTEN activity. Using a pharmacological approach, we demonstrated a role for Src homology 2-containing protein tyrosine phosphatase-1 in the PGE2-induced tyrosine dephosphorylation of PTEN. Collectively, these data reveal that PGE2, via Epac-1 activation, enhances SHP-1 activity, resulting in increased PTEN activity. We suggest that this mechanism contributes to the ability of PGE2 to inhibit PI3K-dependent innate immune signaling in primary macrophages. The Journal of Immunology, 2007, 179: 8530–8536.

Macrophages are key cellular participants in innate immunity, recognizing and ingesting pathogenic microorganisms through both opsonin-dependent and -independent pathways. Among several mechanisms that macrophages use to internalize microbes, FcγR-mediated phagocytosis is an important primary mode of defense in the immune system. Accompanying phagocytosis is the generation of reactive oxygen intermediates and reactive nitrogen intermediates, which facilitate the intracellular killing of ingested pathogens, as well as the production of inflammatory mediators (e.g., cytokines, chemokines, and lipids) that enhance immune responses by recruiting and activating polymorphonuclear leukocytes. These inflammatory responses are teleologically advantageous, but when unregulated or severe, lead to tissue damage with associated morbidity and mortality of the infected host. Thus, these proinflammatory signals must be balanced with counterregulatory anti-inflammatory signals generated during infection to reign in the inflammatory response and ensure its resolution when the microbial threat is cleared. Within the macrophage, a series of complex cascades of reactions is evoked upon ligation and cross-linking of the FcγR, including several mediated by kinases and phosphatase enzymes that dictate the vigor of the subsequent inflammatory response. Recent advances in this area include the recognition that FcγR phagocytosis is negatively regulated by protein Tyr phosphatases as well as inositol phosphates (1–5).

The phagocytic actions of IgG-opsonized targets by the macrophage is initiated when the Fc portion of IgG binds to and clusters the FcγR on the surface of the phagocytic cell (reviewed by Swanson and Hoppe in Ref. 6). This provokes the phosphorylation of FcγR-associated ITAMs by kinases of the Src family, thereby initiating the local assembly of signaling enzymes and adapter-enzyme complexes necessary for successful phagocytosis and the generation of inflammatory mediators (6). One of the crucial participants in FcγR signaling and particle ingestion is PI3K, a lipid kinase that phosphorylates the inositol ring at the 3′ position, generating several products including phosphatidylinositol 3,4,5-trisphosphate (PIP3).4 PIP3 is necessary for the recruitment and activation of pleckstrin homology domain-containing enzymes such as the guanine exchange factor Vav and the Ser/Thr kinase Akt (protein kinase B) (7).

The phagocytic actions of PI3K are countered by the inositol 3-phosphatase and tensin homolog on chromosome 10 (PTEN) (8). PTEN is a protein/lipid phosphatase that can dephosphorylate PIP3 at the D3 position (9). Studies of transfected cells demonstrate that...

4 Abbreviations used in this paper: PIP3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; AM, alveolar macrophage; Epac-1, exchange protein activated by cAMP-1; PKA, protein kinase A; SHP-1, Src homology 2-containing protein tyrosine phosphatase-1; SHPL, α-bromo-4-(carboxymethyl) acetophenone.

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Received for publication April 26, 2007. Accepted for publication October 1, 2007.

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1 This work was supported by National Institutes of Health Grants HL078727 (to D.M.A.) and HL058897 (to M.P.-G.), American Lung Association Research Grant RG8909N (to D.M.A.), Conselho Nacional de Desenvolvimento Científico e Tecnológico (to C.C.), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (to C.C.).

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overexpression of PTEN inhibits FcγR-mediated phagocytosis (10), while primary peritoneal macrophages from PTENnull mice exhibited increased ingestion of IgG-coated targets (8). Additionally, FcγR-induced signaling events such as Akt phosphorylation and ERK phosphorylation were up-regulated in PTENnull macrophages (8). Though these data show that PTEN negatively modulates FcγR phagocytosis, regulators of PTEN activity itself, which might influence FcγR-mediated phagocytosis, remain uncharacterized. It has been reported that PTEN activity can be down-regulated by Tyr, Ser, and Thr phosphorylation (11, 12).

The arachidonic acid metabolite PGE2 is a product of the cyclooxygenase cascade and inducible PGE synthase enzymes, and is a biologically important lipid capable of modulating immune and inflammatory responses (13, 14). PGE2 has potent immunosuppressive properties, including the direct inhibition of leukocyte chemotaxis (15), reactive oxygen intermediate production (16), arachidonic acid release (17), leukotriene synthesis (18), and the generation of myriad proinflammatory cytokines (19). PGE2 has also been reported to be an endogenous suppressor of macrophage FcγR phagocytosis and bacterial killing (20–22). The ability of PGE2 to suppress FcγR phagocytosis and bacterial killing results from its ability to provoke increases in intracellular cAMP following the ligation of heterotrimeric Gαi protein-coupled E prostaglandin receptors (20, 21). Furthermore, we demonstrated in pulmonary alveolar macrophages (AMs) that suppression of phagocytosis by PGE2/cAMP was mediated by the recently described guanine ex-receptors (20, 21). Furthermore, we demonstrated in pulmonary alveolar macrophages (AMs) that suppression of phagocytosis by PGE2/cAMP was mediated by the recently described guanine ex-receptors (20, 21).

Committee for the Use and Care of Animals. PGE2 was obtained from American Scientific Products. Macrophage monolayers were cultured overnight in DMEM with 10% FBS (HyClone). The cells were washed and the medium was changed to DMEM without serum 20 min before challenge with phagocytic targets or use in other assays.

Preparation of erythrocytes and microcolorimetric phagocytosis assay

SRBCs (ICN Pharmaceuticals) were opsonized with a subagglutinating concentration of IgG rabbit anti-sheep erythrocyte Ab (Cappel Organon Teknika) as previously described (31). The phagocytosis of SRBCs by rat AMs was assessed as previously described (20). In other experiments, cells were treated overnight with Lipopolentin reagent (1% v/v), with or without specific Abs against PTEN (mouse monoclonal, clone A2B1; Santa Cruz Biotechnology) or nonspecific mouse monoclonal IgG protein (1:500) in serum-free medium according to the manufacturer’s instructions and previous reports (32). The next day, medium was removed and cells were replenished with fresh serum-free medium. Results are expressed as a percent of the control, to which only vehicle was added.

Immunoprecipitation

For PTEN immunoprecipitation, the macrophage monolayers were washed twice, and lysed in lysis buffer containing protease inhibitors. In these experiments, the AMs were stimulated with 1 μM PGE2, 1 mM Epac-1 agonist, or 1 mM PKA agonist at different time points. In separate experiments, we pretreated the AMs with 100 μM SHPI for 30 min followed by the addition of PGE2, for 15 min. Total protein concentrations were determined using the DC Protein Assay kit (Bio-Rad). Immunoprecipitation was performed on 15 μg of total protein using Protein G-PLUS (Santa Cruz Biotechnology) and 1 μg of anti-PTEN Ab (clone A2B1; Santa Cruz Biotechnology). In experiments for Syk immunoprecipitation, the macrophage monolayers were lysed in buffer containing 1% Triton X-100 containing 50 mM Tris (tris(hydroxymethyl)aminomethane) (pH 8.0), 100 mM NaCl, 1 mM Na3VO4, 1 mM PMSF, 50 mM NaF, and 1 μg/ml leupeptin. Lysates were precleared with protein A-Sepharose for 30 min and incubated overnight at 4°C with anti-Syk (1:80; Santa Cruz Biotechnology). Protein A-Sepharose was added to each sample and incubated for 3 h with rotation at 4°C. The beads were washed briefly three times with lysis buffer without Triton X-100 and proteins were separated on 8% SDS-PAGE gels. The entire volume recovered after boiling the beads was loaded onto the gel. Protein concentration of lysates could not be accurately determined because of interference with the Coomassie protein assay by SDS contained in loading buffer. Thus, lysates are derived from equal numbers of cells, but total Syk or PTEN per lane was subject to variation. The proteins were transferred to nitrocellulose membranes (Schleicher & Schuell) overnight at 100 amps (A) and for 3 h at 200 A.

Immunoblotting

The membrane was blocked with 5% fat-free milk in TBS containing 0.1% Tween 20 for 1 h, washed three times, and then probed with phosphorylation (Ser473) Ab (1:1,000), phospho-p44/42 MAPK (Thr202/Tyr204) Ab (1:1,000), or anti-phospho-Tyr (1:1,000; PY20; BD Transduction Laboratories) for 1.5 h. After that, the membrane was washed and incubated with a HRP-conjugated sheep anti-mouse or HRP-conjugated donkey anti-rabbit secondary Ab (1:15,000; Amersham Pharmacia Biotech). Phosphorylated bands were visualized using the ECL system (Amersham). The membranes were then stripped, blocked, and reprobed with anti-Syk (1:800), anti-p44/42 MAPK (1:1,000), anti-Akt (1:1,000), or anti-PTEN (1:1,000) for 1 h, followed by an incubation with HRP-conjugated donkey anti-rabbit or sheep anti-mouse secondary Ab (Amersham Pharmacia Biotech). The bands were visualized using the ECL system. All nonspecific Abs were obtained from Cell Signaling.

PTEN phosphatase assay

PTEN immunoprecipitates were assessed for phosphatase activity as previously described (33). Briefly, equal volumes of phosphatase reaction buffer containing 200 μM p-nitrophenylphosphate (New England Biolabs) were added to immunoprecipitates and the reaction was allowed to proceed at 37°C for 30 min. Immunoprecipitates were centrifuged and supernatants were added to a 96-well plate in triplicate. BIOMOL Green reagent (BIOMOL Research Laboratories) was added and plates were incubated for 20 min at room temperature. Absorbance of each well was assessed using a colorimeter reader at 630 nm wavelength. In each situation, PTEN activity was quantified as activity observed relative to the control. Results were reported as relative PTEN phosphatase activity ± SEM compared with control conditions.
Bacterial killing assay

The ability of Klebsiella pneumoniae to survive intracellularly following phagocytosis was assessed using a tetrazolium dye (MTT) reduction assay as described elsewhere (34).

Statistical analysis

The data are reported as a representative blot from two or three different experiments. Graphs represent the mean ± SEM from more than or equal to three different experiments. The means from different treatments were compared by ANOVA. When significant differences were identified, individual comparisons were subsequently made with the Bonferroni comparison for unpaired values. Statistical significance was set at a p value ≤0.05.

Results

PGE₂ inhibits FcγR-mediated Akt and ERK1/2 activation, but not Syk activation

To determine whether PGE₂ inhibits the state of activation of downstream targets involved in phagocytic signaling from FcγR, we assessed the phosphorylation/activation of Akt and ERK1/2 during the ingestion of IgG-opsonized SRBCs. Pretreatment of AMs with PGE₂ at concentrations known to suppress FcγR-mediated phagocytosis (20) dose-dependently inhibited both Akt and ERK1/2 activation (Fig. 1, A and B), demonstrating that PGE₂ suppresses the FcγR-mediated activation of signaling molecules regulated by PI3K/PTEN. Because we previously demonstrated that the lipid mediator leukotriene B₄ enhanced FcγR-mediated phagocytosis through the amplification of the phosphorylation/activation of the Tyr kinase Syk (35), we tested whether the inhibitory effect of PGE₂ on FcγR-mediated phagocytosis also involved modulation of Syk activation. Treatment of AMs with PGE₂ did not modify Syk phosphorylation in response to challenge with IgG-opsonized SRBCs (Fig. 1, A and B), ruling out Syk as a target responsible for the inhibitory effect of PGE₂ on FcγR-mediated phagocytosis.

We have previously reported that PGE₂ inhibits fibroblast migration by a mechanism involving an increase in the lipid and Tyr phosphatase activities of PTEN (27). To test the hypothesis that the suppressive effects of PGE₂ on innate immune functions are mediated by PTEN, we treated AMs with the highly selective inhibitor bpV(pic) before exposure to PGE₂ and IgG-SRBCs. As demonstrated by evaluating Akt phosphorylation (Fig. 1C), bpV(pic) prevented the suppression of Akt activation by PGE₂. As a confirmatory approach, we introduced a specific mouse monoclonal anti-PTEN IgG into rat AMs using a liposomal reagent. This Ab, but not a nonspecific mouse IgG, also blocked the actions of PGE₂ on Akt phosphorylation (Fig. 1C). We obtained similar results for ERK 1/2 activation (data not shown). Furthermore, as shown in Fig. 1D, PGE₂ augmented PTEN lipid phosphatase activity in AMs, and this effect could be inhibited by the pharmacological PTEN inhibitor bpV(pic).

The inhibition of FcγR-mediated phagocytosis by PGE₂ is PTEN dependent

We next examined the involvement of PTEN in the inhibition of FcγR-mediated phagocytosis by PGE₂ by seeking to block PTEN using either bpV(pic) or the specific anti-PTEN IgG-based method. Rat AMs were pretreated with bpV(pic) (100 nM), the anti-PTEN IgG, or a nonspecific IgG before the addition of PGE₂ (1 μM, 5 min) and subsequently challenged with IgG-SRBCs. PGE₂-mediated inhibition of phagocytosis was largely suppressed by bpV(pic) treatment (Fig. 2A) or the PTEN-specific IgG (Fig. 2B). Taken together, these data suggest that the inhibitory effect of PGE₂ on FcγR-mediated phagocytosis depends on PTEN activity.

PTEN mediates the inhibition of FcγR-mediated phagocytosis by Epac-1

The inhibition of AM phagocytosis by PGE₂ results from its ability to increase intracellular cAMP (20) with the subsequent activation of the guanine exchange factor Epac-1 (21). Therefore, we examined whether PTEN activity mediates the suppression of phagocytosis by Epac-1 activation as it does for PGE₂. AMs were pretreated for 30 min with or without 100 nM bpV(pic) before incubation with the highly selective Epac-1 agonist 8-pCPT-2′-O-Me-cAMP (1 nM, 60 min) and subsequent challenge with IgG-SRBCs. As shown in Fig. 3A, Epac-1-mediated inhibition of phagocytosis was largely suppressed by bpV(pic). Similar to what

FIGURE 1. PGE₂ inhibits FcγR-mediated Akt and ERK1/2 activation, but not Syk activation, via enhanced PTEN activation. A, AMs were pretreated with the indicated concentrations of PGE₂ for 2 min before the addition of IgG-SRBCs (1:100 ratio) and then incubated for 7 (for Syk assay) or 20 (Akt and ERK-1/2) min at 37°C. Incubations were terminated by the addition of lysis buffer, and lysates were subjected to immunoprecipitation and/or immunoblotting as described in Materials and Methods. Immunoblots in upper panels represent phosphorylated forms of Syk, Akt, and ERK-1/2 detected with anti-phosphotyrosine Ab, specific anti-phospho Akt, or anti-phospho ERK-1/2, and those in lower panels, the total amounts of those proteins evaluated with anti-Syk, anti-Akt, or anti-ERK-1/2 Abs, respectively. Results are representative of three separate experiments with mean band densitometry values shown beneath each band, expressed relative to the IgG-SRBC treated control. B, The relative degree of inhibition of phosphorylation (activation) of Syk, Akt, and ERK-1 with the maximal (1 μM) concentration of PGE₂ is depicted graphically (mean ± SEM), **, p < 0.01, *** p < 0.001 compared with the IgG-SRBC treated control as determined by ANOVA. C, AMs were pretreated with the specific PTEN inhibitor bpV(pic) (100 nM × 30 min), a mouse monoclonal anti-PTEN IgG, or a nonspecific mouse IgG as detailed in Materials and Methods. Cells were then treated with PGE₂ (1 μM, 2 min) before the addition of IgG-SRBCs (1:100 ratio) and then incubated for 20 min at 37°C. Cells were lysed and assessed for Akt phosphorylation (data not shown). Mean relative band densitometry data (n = 2) are shown beneath each band. D, AMs were pretreated with bpV(pic) (100 nM), or vehicle for 15 min followed by PGE₂ (1 μM) for 15 min. PTEN activity was determined as described in Materials and Methods. Results represent the mean (± SE) for three independent experiments performed in quadruplicate. *, p < 0.05, **, p < 0.01, and ***, p < 0.001 vs control by ANOVA.
was observed for PGE₂ (Fig. 2B), the Epac-1 agonist also significantly enhanced PTEN activity (Fig. 3B). The selective protein kinase A (PKA) activating analog 6-Bnz-cAMP, which did not inhibit FcγR phagocytosis in AMs (21), slightly stimulated PTEN lipid phosphatase activity, though its effect was not as great as that of the Epac-1 agonist (Fig. 3B).

PTEN mediates the inhibition of bacterial killing by PGE₂

We previously reported that PGE₂ inhibits the intracellular killing of immune serum-opsonized Klebsiella pneumoniae in the AM (21), a model that depends on the FcγR for phagocytosis of the bacterium (36). We therefore speculated that PTEN might also play a role in mediating the suppression of bacterial activity by PGE₂ in these cells. To address this hypothesis, we pretreated AMs for 30 min with bpV(pic) at 20 and 100 nM before exposure to PGE₂ or vehicle. As shown in Fig. 4A, bpV(pic) treatment significantly reduced the survival of ingested bacteria (i.e., enhanced the bacterial killing capacity) in PGE₂-unexposed cells, suggesting that PTEN functions as an endogenous brake on such activity. Furthermore, the effect of PGE₂ on killing was reversed by this inhibitor as well (Fig. 4B), suggesting a dependence on PTEN for its suppression of bactericidal activity.

PGE₂ and Epac-1 activation cause the Tyr dephosphorylation of PTEN

We next explored the state of phosphorylation of PTEN on Tyr residues, which is inversely correlated with PTEN activity (11). In untreated cells, basal Tyr phosphorylation of PTEN was observed. PGE₂ treatment lead to a time-dependent Tyr dephosphorylation of PTEN in AMs (81 ± 19% maximal dephosphorylation by 30 min) (Fig. 5A). Epac-1 activation by its specific agonist lead to a more rapid dephosphorylation of PTEN, with a maximal effect (67 ± 7% dephosphorylation) seen within 5 min of its addition (Fig. 5A). Reflecting its ability to only slightly enhance PTEN lipid phosphatase activity, the PKA agonist 6-Bnz-cAMP suppressed PTEN phosphorylation, but to a lesser degree than either PGE₂ treatment or direct Epac-1 activation (45 ± 5% dephosphorylation by 30 min) (Fig. 5A). These data suggest that PGE₂ enhances PTEN activity by promoting its dephosphorylation, and also support the observation from Fig. 3B that this is likely mediated by activation of Epac-1 to a greater extent than by activation of PKA.

The Tyr phosphatase SHP-1 mediates dephosphorylation of PTEN in response to PGE₂ treatment

We hypothesized that the Tyr phosphatase SHP-1 might participate in the regulation of PTEN by cAMP-elevating stimuli such as PGE₂ because 1) the overexpression of SHP-1 was previously
found to negatively regulates FcγR-mediated phagocytosis (37) and 2) SHP-1 has been postulated to be capable of dephosphorylating PTEN in a transfected cell system (11). To test this possibility, we pretreated AMs with the SHP-1 inhibitor SHPI (100 μM, 30 min) or vehicle, then incubated the cells in the presence or absence of PGE2 (1 μM, 15 min) before assessing PTEN tyrosine phosphorylation. To ensure equal protein loading, blots were stripped and reprobed for PTEN (lower panels). The blots are representative of three independently performed experiments. Densitometry for the ratio of pY:PTEN was performed as described in the Materials and Methods and normalized to the untreated condition. Shown are the mean values from three independent experiments.

Discussion

In the present study, we extended our exploration of the mechanisms by which PGE2 modulates AM signaling, phagocytosis, and killing by focusing on its ability to modulate phosphatase activity. Our data provide novel insights into the mechanism by which PGE2 inhibits the antimicrobial actions of lung phagocytes. We demonstrate that PTEN, which antagonizes the stimulatory effects of PI3K, is a key target for the negative modulatory effects of this prostanooid on both phagocytosis and bacterial killing. PGE2 enhanced the lipid phosphatase activity of PTEN, which was due to its Tyr dephosphorylation. The dephosphorylation appears to be mediated by the Tyr phosphatase SHP-1. Further studies are needed to elucidate the mechanisms by which PGE2 activates SHP-1, but it appears that the cAMP effector Epac-1 participates in this process. Our data corroborate the finding that SHP-1 inhibits FcγR-mediated phagocytosis (5). The proposed mechanisms by which PGE2, cAMP, Epac-1, SHP-1, and PTEN interrelate in suppressing FcγR-mediated phagocytosis and killing are depicted in the model illustrated in Fig. 6.

Brockdorff et al. (38) have proposed that activated SHP-1 is involved in the dephosphorylation of Zap70 and Syk, and in subsequent inhibition of TCR signaling. In contrast to that fact, our data support the hypothesis that Syk is not a target for the negative modulatory effect of PGE2, because no inhibitory effect on FcγR-mediated Syk activation was observed in the presence of PGE2. These observations instead suggest mechanisms of SHP-1 action which are context dependent.

In the present study, we verify that signals downstream of PI3K, such as Akt and ERK-1/2, were dose-dependently inhibited by PGE2 treatment, suggesting that the effect of this prostanooid precedes or acts at the level of PI3K. PTEN involvement was therefore considered. A pivotal role for PTEN as a mediator of the suppressive effects of PGE2 was supported by the facts that PTEN phosphatase activity was increased by PGE2 and that PTEN inhibition abrogated the inhibitory effects of PGE2 on both FcγR-dependent phagocytosis as well as Akt/ERK activation.

Recently, phosphodiesterase-resistant cAMP analogs that are highly selective in their activation of either PKA or Epac-1 have been developed (39). We used the best characterized of these compounds, the PKA activator 6-Bnz-cAMP and the Epac-1 activator 8-pCPT-2’-O-Me-cAMP (39). These compounds exerted different effects on phagocytosis at the indicated concentrations as previously described (21). The Epac-1 activator exhibited a profile of actions (stimulation of PTEN activity, dephosphorylation of PTEN, and inhibitory effects on phagocytosis which were abrogated by the PTEN inhibitor) which more closely resembled that of PGE2 than did the PKA activator. In this respect, it is interesting that although selective PKA activation failed to inhibit FcγR phagocytosis in rat AMs (21), 6-Bnz-cAMP both increased PTEN activity and PTEN dephosphorylation, although with lesser potency than either PGE2 or 8-pCPT-2’-O-Me-cAMP. This discrepancy between PKA activation and the regulation of phagocytosis might be due to any of several possible explanations. We speculate that the degree of PTEN activation by PKA activation was simply insufficient to suppress FcγR-mediated ingestion. It is also possible that the intracellular location of these different effectors may be important and we did not address this in the present study. Finally, PKA activation might itself trigger signals that interfere with SHIP-1/PTEN signaling cascades such that the
two opposing effects cancel each other out. This possibility is sug-
ggested by the fact that we and others have found that the inhibition of
PKA suppresses FcγR-mediated phagocytosis (21, 40).

The intracellular regulation of PTEN activity is incompletely
understood, but is dependent in part on Tyr phosphorylation, C-
terminal Ser and Thr phosphorylation, membrane localization via
the C2 domain, and ubiquitination and proteasome degradation
(11, 12, 41, 42). The multiple means by which PTEN activity is
regulated are typical for a critically important determinant of cell
function, and present a range of possibilities by which stimuli
might modulate its activity. Because SHP-1 has been reported to
selectively bind and dephosphorylate PTEN in transfected tumor
cells (11), we evaluated the role of SHP-1 in PGE2-induced PTEN
dephosphorylation by using a SHP-1 inhibitor. In this context, we
demonstrated that SHP-1 plays a pivotal role in PTEN dephos-
phorylation evoked by PGE2 (Fig. 5). We were unable to use SHPI
to block the effect of PGE2 in our assay of FcγR-mediated phago-
cytosis because SHPI itself inhibited phagocytosis (data not
shown), an unexpected finding suggesting that a degree of SHP-1
activity may be required for proper phagocytosis via FcγR.

Our conclusions regarding the role of SHP-1 in our model are
limited by the use of SHPI. The compound SHPI is part of a
class of protein tyrosine phosphatase inhibitors that possess
some activity against protein tyrosine phosphatase 1B as well as
SHP-1 (43); therefore, we cannot absolutely exclude a role for
protein tyrosine phosphatase 1B in our system. However, SHPI
has not been reported to inhibit PTEN and does not inhibit
alkaline or acid phosphatases at concentrations up to 10 mM
(43). We used this agent at a concentration of 100 μM because
this compound was found to have a dissociation constant (Kd) of
~200 μM against the catalytic site of SHP-1 (43) and has been
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In summary, we demonstrate for the first time that PGE2 in-
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This limits PI3K signaling, resulting in a suppression of down-
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Disclosures
The authors have no financial conflict of interest.

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