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The Coordination of Prostaglandin E_2 Production by Sphingosine-1-phosphate and Ceramide-1-phosphate

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ABSTRACT

The ability of pro-inflammatory cytokines such as interleukin-1β (IL-1β) to induce the major inflammatory mediator prostaglandin (PG) E_2 depends on the activation of two rate-limiting enzymes, phospholipase A_2 (PLA_2) and cyclooxygenase 2 (COX-2). PLA_2 acts to generate arachidonic acid, which serves as the precursor substrate for COX-2 in the metabolic pathway leading to PGE_2 production. However, less is known about the mechanisms that coordinate the regulation of these two enzymes. We have provided prior evidence that sphingosine kinase 1 and its bioactive lipid product sphingosine-1-phosphate (S1P) mediate the effects of cytokines on COX-2 induction, whereas ceramide kinase and its distinct product, ceramide-1-phosphate (C1P), are required for the activation and translocation of $cPLA_2$ (*FASEB J* **17:**1411–1421. 2003; *J Biol Chem* **278:**38206–38213, 2003; *J Biol Chem* **279:**11320–11326, 2004). Herein, we show that these two pathways are independent but coordinated, resulting in synergistic induction of PGE_2. Moreover, the combination of both S1P and C1P recapitulates the temporal and spatial activation of $cPLA_2$ and COX-2 seen with IL-1β. Taken together, the results provide, for the first time, a mechanism that assures the coordinate expression and activation in time and space of COX-2 and $cPLA_2$, assuring maximal production of PGE_2.

Pro-inflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), regulate both the type IVA cytosolic PLA_2 ($cPLA_2$) and the inducible cyclooxygenase 2 (COX-2) enzyme to affect prostaglandin production. The action of these cytokines involves distinct mechanisms such that COX-2 is regulated at the level of transcription and/or message stability (Hinz and Brune, 2002; Simmons et al., 2004), whereas $cPLA_2$ is regulated post-translationally through less well defined signaling mechanisms leading to membrane translocation (Lin et al., 1993; Hirabayashi and Shimizu, 2000). Recent studies have begun to implicate sphingolipids in the regulation of both $cPLA_2$ and COX-2 as well as of other targets important in inflammation and/or cancer pathogenesis and therapeutics (Pettus et al., 2003a,b, 2004; Ogretmen and Hannun, 2004; Baumruker et al., 2005; Billich et al., 2005). Thus, it was shown that the ability of TNF-α to induce COX-2 was abrogated by the down-modulation of SK1, and this was reconstituted by the addition of S1P (Pettus et al., 2003b). Therefore, the SK1/S1P pathway seems to be required upstream of the induction of COX-2.

An independent line of investigation implicated ceramide kinase (CK1) and its product C1P in the activation of $cPLA_2$ in response to IL-1β. These results demonstrated that IL-1β led to activation of CK1, resulting in the formation of C1P. Furthermore, C1P was demonstrated to directly bind and activate $cPLA_2$ in vitro and in cells, whereas down-regulation of CK1 was shown to prevent the activation of $cPLA_2$ in response to IL-1β (Pettus et al., 2003a, 2004). These studies also demonstrated that either SK1 RNAi or CK1 RNAi was sufficient to inhibit PGE_2 production in response to cytokines

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ABBREVIATIONS: IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic phospholipase A_2; COX-2, cyclooxygenase 2; S1P, sphingosine-1-phosphate; SK1, sphingosine kinase 1; CK1, ceramide kinase 1; PBS, phosphate-buffered saline; RNAi, RNA interference; AA, arachidonic acid; siRNA, small-interfering RNA; SCR, scrambled RNAi.

by nearly 80% (Pettus et al., 2003a,b, 2004), suggesting that both SK1 and CK1 are necessary for PGE_2 formation.

Given these considerations, it became important to determine whether CK1 and SK1 acted in series or regulated their respective targets ($cPLA_2$ and COX-2) independently. We therefore address herein the independent nature of SK1 and its lipid product S1P from CK1 and its lipid product C1P on COX-2 and $cPLA_2$. In addition, because both $cPLA_2$ and COX-2 are rate-limiting for PGE_2 production, we reproduce the coordinated regulation of these enzymes through their respective sphingolipid signaling components with resultant synergistic increase in PGE_2 production.

Materials and Methods

Cell Lines and Culture Conditions. A549 cells were maintained in 5% fetal calf serum/Dulbecco's modified Eagle's medium with 60 mg/ml kanamycin sulfate at 37°C in a 5% CO_2 incubator. For most assays, cells were plated at 1×10^5 cells/ml for 24 h. After washing with phosphate-buffered saline (PBS), cells were changed to 2% Dulbecco's modified Eagle's medium and returned to standard incubator conditions 2 h before addition of agonists. For RNAi studies, cells were seeded at 0.5×10^5 to allow for 36 h of growth. In all cases, cells were treated at 60 to 80% confluence.

Release of Arachidonic Acid and Metabolites. For quantification of AA release, A549 cells (5×10^4) were labeled overnight with 5 μCi/ml arachidonic acid (5 nM) and washed and treated as described previously (Pettus et al., 2003a).

Measurement of PGE_2 Levels. Media were taken at indicated time points and assayed according to manufacturer's instructions using the prostaglandin E_2 monoclonal enzyme immunoassay kit from Cayman Chemical (Ann Arbor, MI). In brief, media containing PGE_2 competes with PGE_2-acetylcholinesterase conjugate for a limited amount of PGE_2 monoclonal antibody. The antibody-PGE_2 conjugate binds to a goat-anti-mouse antibody previously attached to the wells. The plate is washed to remove any unbound reagents, and then the substrate to acetylcholinesterase is provided.

Immunoblotting. Lysates were normalized to protein concentration using a protein assay reagent (Bio-Rad, Hercules, CA). Each sample (20 μg) was resolved on 7.5% polyacrylamide gels (SDS-polyacrylamide gel electrophoresis) under denaturing conditions and then transferred to 0.45-μm nitrocellulose membranes. After blocking overnight at 4°C with 5% nonfat milk in Tris-buffered saline/0.01% Tween 20 and washing, the membranes were incubated with the relevant antibodies for 3 h at room temperature. The membranes were washed extensively in Tris-buffered saline/0.1% Tween 20. The blots were probed using specific anti-human COX-2 polyclonal primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). Bands were visualized using the appropriate horseradish peroxidase-conjugated secondary antibody and the enhanced chemiluminescence immunoblotting detection system (Amersham Biosciences, Piscataway, NJ). In all cases, equal loading was verified by staining with 0.1% Amido black in methanol/acetic acid/water. Measurements were performed using densitometry software with controls from each gel normalized to allow equal baseline comparisons for -fold change determinations with IL-1β treatment with respect to respective RNA interference controls.

Immunofluoresence. Cells were fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. Fixed cells were permeabilized with 0.1% Triton X-100 for 10 min at room temperature and blocked for 1 h with PBS containing 2% human serum. Cells were immunostained with goat anti-human $cPLA_2$ (10 μg/ml; Santa Cruz Biotechnology) and mouse anti-human COX-2 (10 μg/ml; BD Transduction Laboratories, Lexington, KY) in PBS containing 2% human serum and incubated for 2 h at room temperature. After extensive washing with PBS, cells were treated with tetramethylrhodamine B isothiocyanate-conjugated anti-goat IgG or Alexa488-conjugated anti-mouse IgG in PBS containing 2% human serum for 1 h at room temperature. Localization of COX-2 or $cPLA_2$ were visualized by confocal microscopy (LSM 510; Carl Zeiss, Inc., Thornwood, NY).

$cPLA_2$ Translocation Measurements. At the time points shown, cells were fixed and immunostained for $cPLA_2$ as described above, and three fields were counted at 60× for an approximate sampling of 50 to 100 cells for each determination. Cells were counted as translocated if $cPLA_2$ was predominantly in a perinuclear endoplasmic reticulum/Golgi distribution as opposed to a diffuse cytoplasmic distribution. The percentage of the total cells with demonstrable translocation is presented as percentage of control.

Statistical Methods. Student's t test was performed between control and treated states and/or between treatment and treatment plus RNAi-mediated inhibition states on a minimum of three independent experiments (in some cases performed in duplicate or triplicate as described in the figure legend).

RNA Interference. Gene silencing of human SK1 and CK1 were performed as described previously (Pettus et al., 2003a,b) using sequence specific siRNA reagents and controls (QIAGEN). All sequences were evaluated against the database using the National Institutes of Health blast program to test for specificity. A549 cells were transfected with the 21-nucleotide duplexes using OligofectAMINE (Invitrogen) as recommended by the manufacturer. After 36 h, cells were treated with IL-1β and assayed as described.

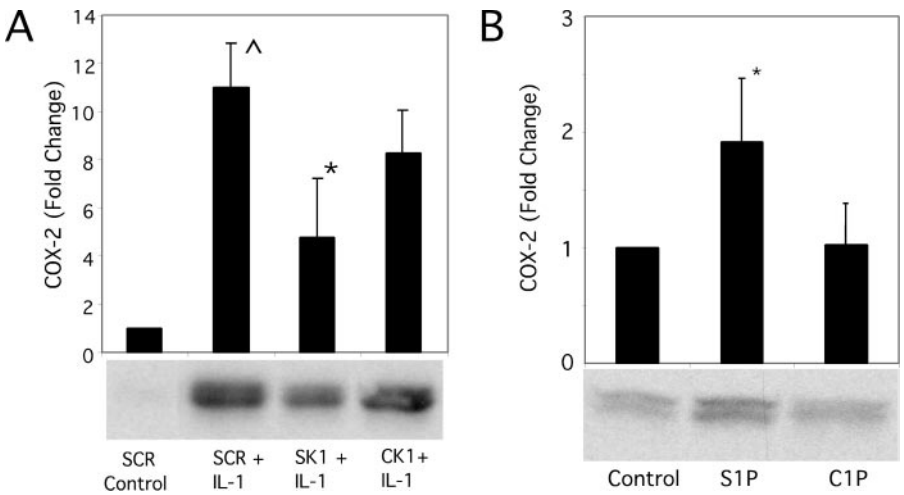

Fig. 1. The SK1/S1P pathway but not the CK1/C1P pathway regulates IL-1β–mediated COX-2 induction. A549 cells were treated with or without (Control) IL-1β (2.5 ng/ml) in the presence or absence of 200 nM SCR RNAi or RNAi to SK1 or CK1, and the -fold change in COX-2 protein with respect to RNAi controls was measured at 4 h (A). IL-1β induced ($\wedge$, $P = 0.01$) 11-fold induction of COX-2, whereas SK1 but not CK1 RNAi significantly inhibited IL-1β induction (*, $P = 0.02$) of COX-2. B, COX-2 induction is noted relevant to vehicle control (Control) in response to 2 h of 1 μM S1P delivered in 0.098% ethanol/0.002% dodecane but not 1 μM C1P treatment. All data are representative of at least three experiments, each analyzed in triplicate, and are presented as the average ± S.E.

Results

To determine whether CK1 and SK1 acted in series or regulated their respective targets ($cPLA_2$ and COX-2) independently, we first set out to evaluate the specificity of these signaling pathways for what was previously determined to be their respective targets (Pettus et al., 2003a,b, 2004). Thus, we first sought to determine whether IL-1β induced COX-2 and whether this induction was dependent on SK1 and/or CK1. Cells were treated with 200 nM concentrations of either scrambled (SCR), SK1, or CK1 RNAi for 36 h, and then treated with IL-1β for 4 h. The levels of COX-2 were evaluated by immunoblotting. As shown in Fig. 1A, IL-1β induced COX-2 11-fold above an appreciable baseline level by 4 h, and SK1 RNAi inhibited this induction by 65%. It is noteworthy that CK1 RNAi did not significantly inhibit the induction of COX-2 by IL-1β. In addition, we checked sphingosine kinase activity upon IL-1β stimulation and found that IL-1β induced a 1.49-fold increase in sphingosine kinase activity (average of triplicate determinations) at 10 min, consistent with our previous results with TNF-α in L929 and A549 cells and the findings of others using IL-1β (Pitson et al., 2000; Pettus et al., 2003b; Billich et al., 2005). Free sphingosine was also significantly decreased in IL-1β–stimulated cells at 10 min from 34.7 pmol/mg (average of triplicate determinations) protein to 25.0 pmol/mg of protein (average of triplicate determinations). Thus, IL1 activates SK1, and SK1 (but not CK1) is necessary for COX-2 induction.

Next, to determine whether either of the two lipid products was sufficient to induce COX-2, cells were treated with S1P or C1P (1 μM), and the levels of COX-2 were determined by immunoblot analysis. S1P but not C1P significantly elevated COX-2 protein (Fig. 1B). Taken together, these results show that the SK1/S1P pathway is necessary and sufficient for the induction of COX-2, whereas the CK1/C1P pathway seems not to play a significant role in this response.

By contrast, it has been demonstrated that the CK1/C1P pathway is involved in IL-1β–mediated induction of arachidonic acid release. In addition, C1P has been shown to stimulate $cPLA_2$ in vitro and to induce its translocation to perinuclear regions involving the Golgi apparatus (Pettus et al., 2003a, 2004). Thus, it became important to determine whether SK1 affected this specific response. IL-1β–mediated activation of arachidonic acid release and $cPLA_2$ activation was, therefore, measured with and without RNAi to the respective sphingolipid signaling pathways. It is interesting that whereas RNAi to CK1 inhibited activation and translocation of $cPLA_2$, RNAi to SK1 had no such effect (Fig. 2, A and B). Thus, the regulation of $cPLA_2$ in response to IL-1β is

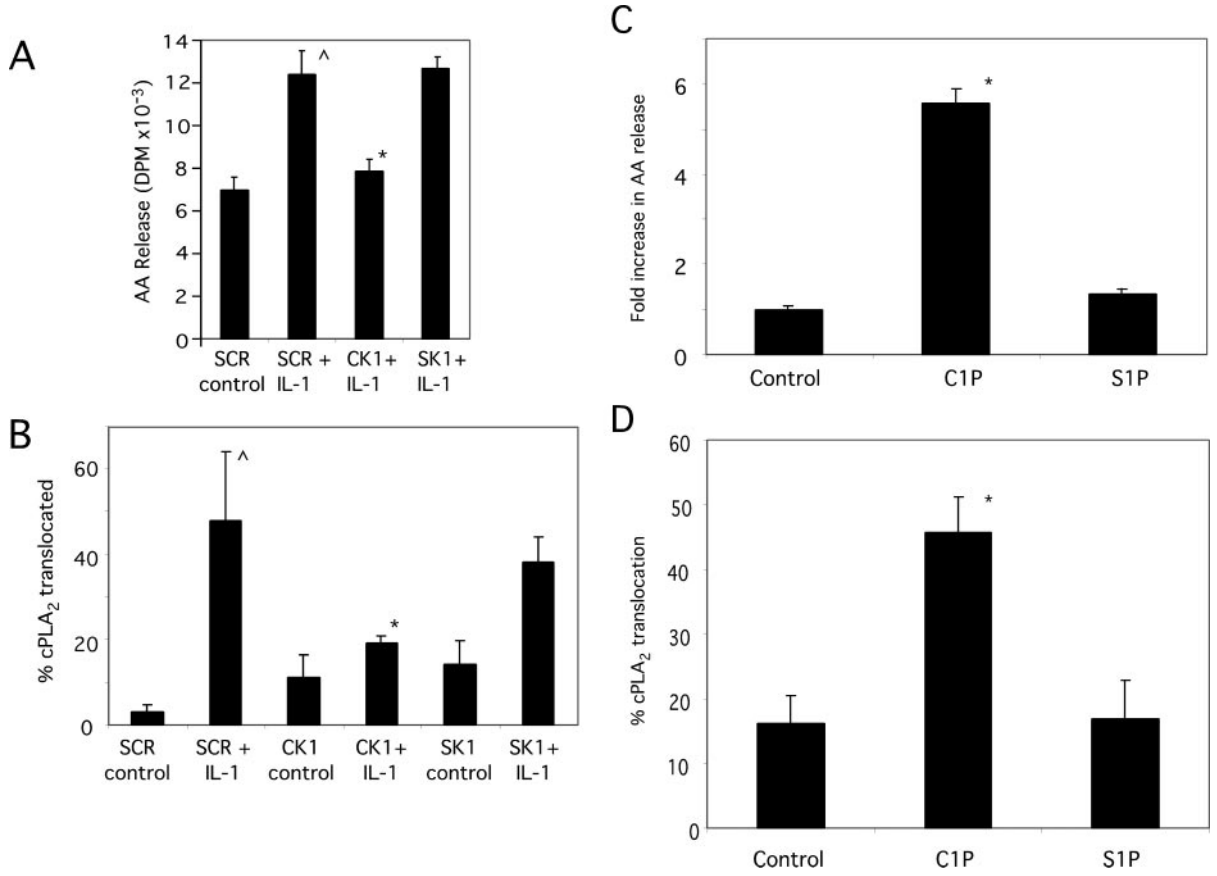

Fig. 2. The CK1/C1P pathway but not the SK1/S1P pathway regulates IL-1β mediated $cPLA_2$ activation/translocation. A549 cells were treated with or without (Control) IL-1β (2.5 ng/ml) in the presence or absence of SCR RNAi or RNAi to SK1 or CK1 and the change in AA release (A) and translocation of $cPLA_2$ (B) were measured at 4 h. IL-1β induced significant release of AA ($\wedge$, $P = 0.01$) (A) and translocation of $cPLA_2$ ($\wedge$, $P = 0.02$) (B), whereas CK1 but not SK1 RNAi significantly inhibited AA release (*, $P = 0.02$) (A) and $cPLA_2$ translocation (*, $P = 0.04$) (B). A549 cells were treated with vehicle control or 1 μM S1P and C1P (C and D). Statistically significant AA release (*, $P < 0.0001$) was noted with (1 μM) C1P in 0.098% ethanol/0.002% dodecane but not S1P after 4 h (C). In addition, statistically significant $cPLA_2$ translocation (*, $P < 0.005$) was also noted with (200 nM) C1P but not S1P after 4 h (D). The data are representative of at least three experiments performed, and in the case of the AA release assay, were analyzed in at least triplicate. Data presented as average $\pm$ S.E.

dependent on CK1 but not SK1, suggesting that formation of S1P is not upstream of ceramide kinase and $cPLA_2$ activation in response to IL-1β.

The two bioactive sphingolipids also demonstrated selective effects on activation of $cPLA_2$. Thus, we found that exogenous C1P but not S1P activated $cPLA_2$, as demonstrated by measured AA release (Fig. 2C) and $cPLA_2$ translocation (Fig. 2D). Therefore, and in contradistinction with the results on COX-2 induction, the activation of the $cPLA_2$ requires the CK1/C1P pathway and not the SK1/S1P pathway.

Because the above results showed that C1P and S1P independently regulate two distinct downstream effects of IL-1β, we hypothesized that C1P and S1P may act synergistically for maximal PGE_2 production, with C1P releasing the AA substrate and S1P enhancing the transformation of AA to PGE_2 through induction of COX-2. It should be noted that the action of IL-1β displays distinct temporal patterns on the key elements in PGE_2 production, as demonstrated previously (Pettus et al., 2003a,b, 2004). Thus, IL-1β induces COX-2 protein, $cPLA_2$ translocation, and PGE_2 production after 2 to 4 h, whereas S1P is generated at 10 min and C1P at 3.5 to 4.0 h (Pettus et al., 2003a,b, 2004).

Therefore, A549 cells were treated in such a manner as to mimic the kinetics of S1P and C1P formation in response to IL-1β. Cells were treated with S1P (or vehicle as control); after 2 h, the cells were treated with C1P. The production of PGE_2 was then evaluated 2 h later (4 h after the initial treatment with S1P). As shown in Fig. 3A, these lipids displayed significant synergy on PGE_2 production, even at concentrations as low as 0.3 μM S1P and 0.3 μM C1P, which had modest effects on their own. In addition, we continued to find additive enhancement of PGE_2 production even at concentrations previously shown to be maximally effective for each independent pathway, namely 1 μM C1P and 3 μM S1P (data not shown). Based on these findings, we set out to determine the magnitude and range of this synergistic interaction. Thus, each lipid was added to cells at 0, 0.01, 0.03, 0.1, 0.3, 1, 3, and 5 μM alone and with increasing concentrations of the other lipid. The effective dose for 50% maximal activation (ED_{50}) was next determined for each S1P and C1P alone and with addition of various concentrations of the other lipid with the intent of graphically determining an isobologram (Li et al., 1993). Fig. 3B shows the resultant data points displayed with a line from the ED_{50} of S1P to that of C1P, representing the expected location of the data should the interaction have proven additive. On the other hand, isobolic and hyperbolic curves represent interactions that are synergistic or antagonistic, respectively. As shown in Fig. 3B, profoundly isobolic ED_{50} curves result when both S1P and C1P are added in combination as described above. We can therefore conclude

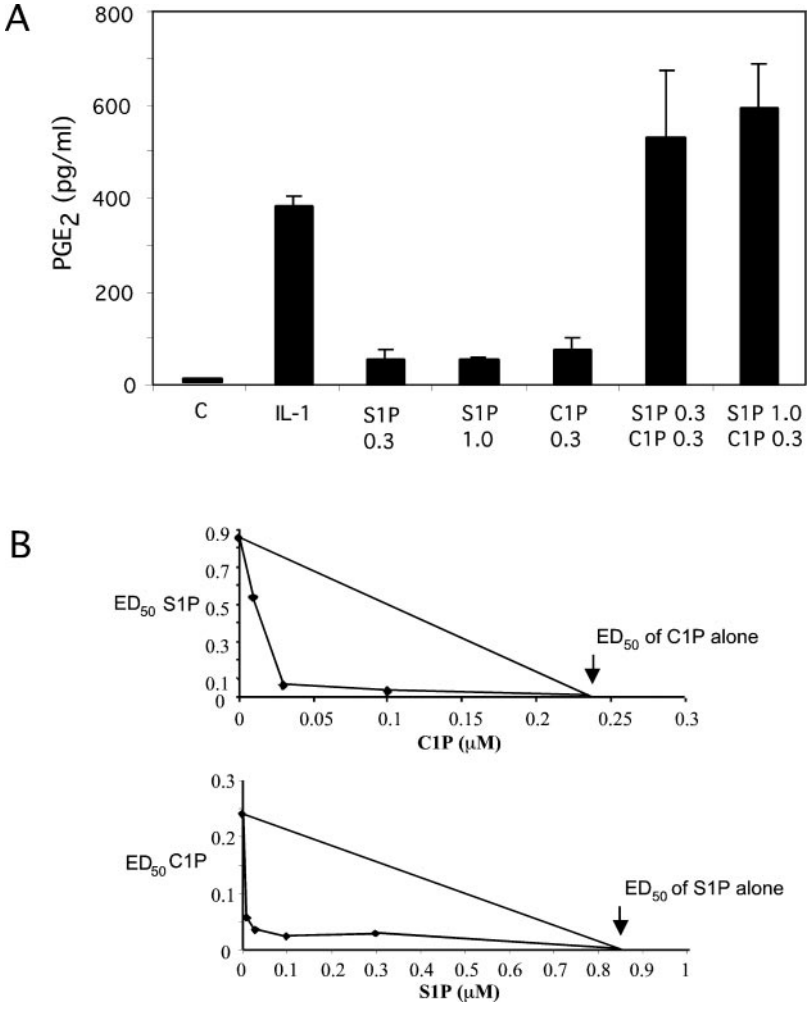

Fig. 3. Synergistic effects of S1P and C1P on PGE_2 production. A549 cells were treated with IL-1β (2.5 ng/ml) and in the presence or absence of vehicle control (Control) or 0.3 or 1.0 μM (A) or 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, or 5.0 μM (B) concentrations of S1P and/or C1P in 0.098% ethanol/0.002% dodecane. S1P was added for 2 h followed by an additional 2 h with C1P. Media were taken and assayed for PGE_2 as described under *Materials and Methods*. Data in A are representative of duplicate experiments analyzed in triplicate and presented as average ± S.E. Data in B are representative of duplicate experiments with ED_{50} values for the entire concentration range plotted against the respective dose addition of each S1P and C1P.

that S1P and C1P act independently and synergistically for maximal PGE_2 production.

Given the kinetics of the IL-1β effects on $cPLA_2$ translocation and induction of COX-2, we wondered whether the action of IL-1β leads to simultaneous colocalization of $cPLA_2$ with newly induced COX-2. In addition, we wondered whether the actions of S1P and C1P reproduced the pattern induced by IL-1β. Control cells expressed appreciable COX-2, and $cPLA_2$ showed a diffuse cytosolic pattern (Fig. 4A). Treatment of cells with IL-1β resulted in induction of COX-2 and its localization to the perinuclear and Golgi areas of the cell (Fig. 4E). IL-1β treatment primarily resulted in translocation of $cPLA_2$ to a perinuclear region and Golgi apparatus with significant enhanced partial colocalization with COX-2. Thus, IL-1β induced a redistribution and induction of $cPLA_2$ and COX-2, respectively, within a similar time frame. Next, it became critical to determine whether exogenous S1P and C1P recapitulate these effects of IL-1β. To mimic the kinetics of endogenous elevations of S1P and C1P in response to IL-1β and the timeline of COX-2 induction and $cPLA_2$ redistribution, A549 cells were treated first with S1P for 2 h followed by C1P for an additional 2 h. The results show that S1P (Fig. 4B), but not C1P (Fig. 4C), induced COX-2 expression, whereas C1P but not S1P induced $cPLA_2$ translocation (Fig. 4, A–C). Furthermore, treatment with the two lipids caused a partial colocalization of the two enzymes in the perinuclear regions that significantly reproduced the pattern of IL-1β treatment alone (Fig. 4D).

Discussion

The results reported here provide specific insights on mechanisms by which novel sphingolipid signaling pathways are involved in the regulation of key rate-limiting steps, namely $cPLA_2$ and COX-2, which in turn are required for the

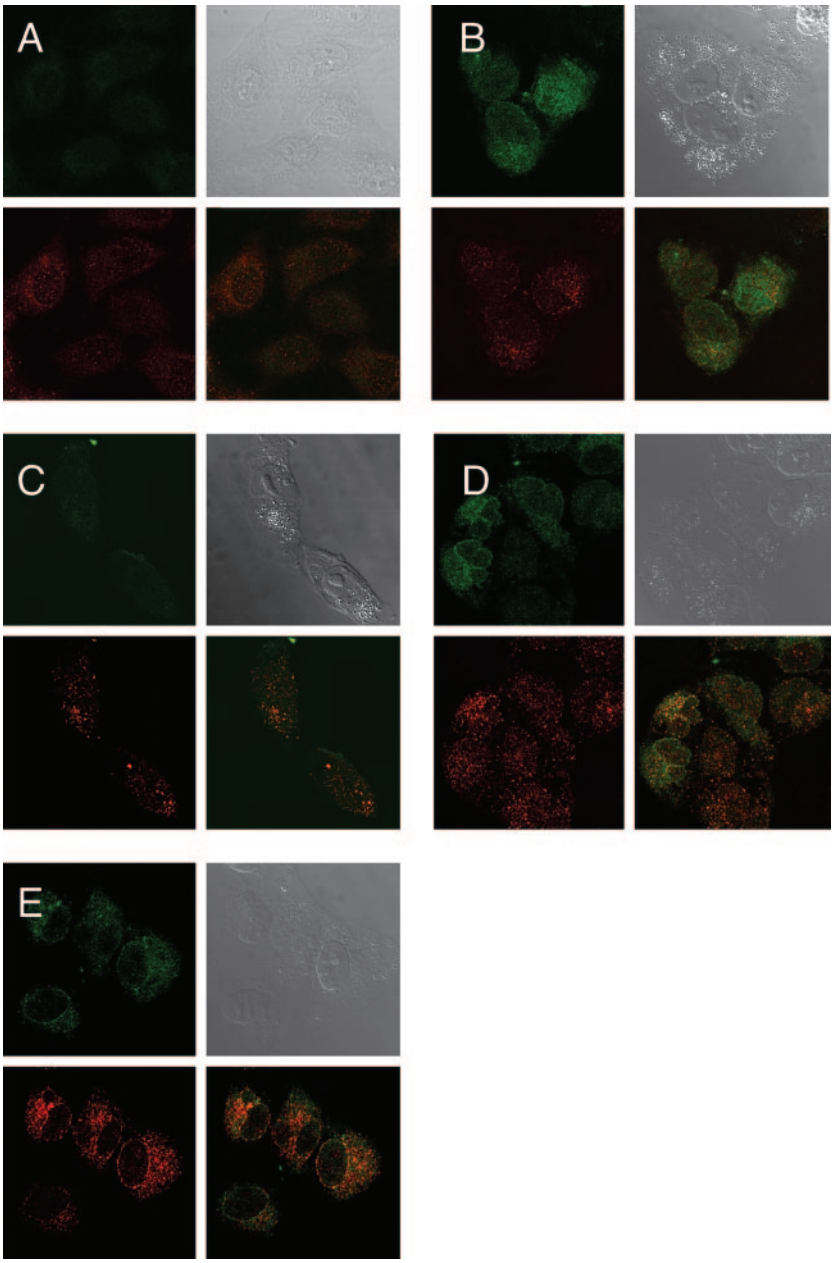

Fig. 4. Effects of IL-1β, S1P, and C1P on colocalization of $cPLA_2$ and COX-2. A549 cells were treated with vehicle (A) or with 200 nM S1P (B), 200 nM C1P (C), both lipids (D), or 2.5 ng/ml IL-1β (E). In D, S1P was added for 2 h followed by an additional 2 h with C1P, whereas in E, cells were treated with IL-1β for 4 h. Cells were immunostained for COX-2 (green) and $cPLA_2$ (red). Areas of colocalization of anti-COX-2 with $cPLA_2$ are in yellow. Each panel consists of four parts, showing COX-2 (top left), $cPLA_2$ (bottom left), the merged images (bottom right), and the phase-contrast images (top right).

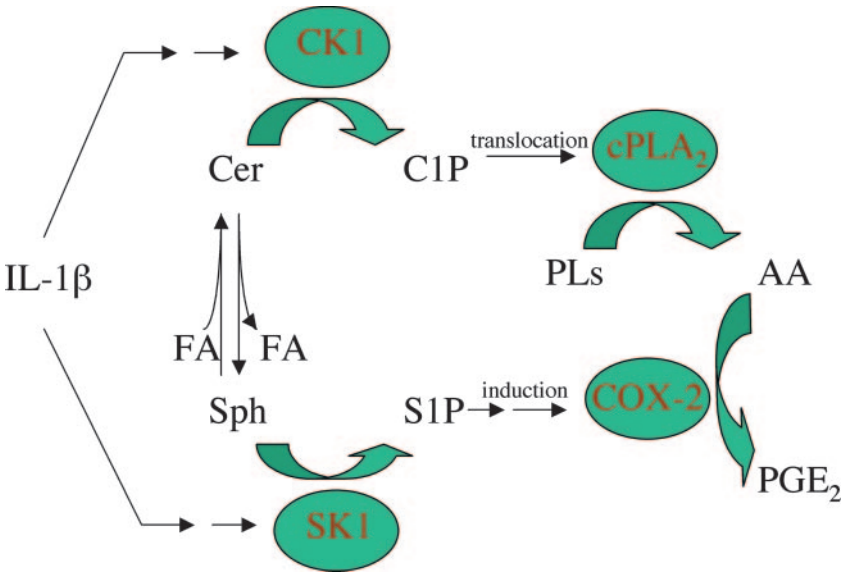

Fig. 5. Proposed mechanisms of sphingolipid involvement in AA production and metabolism. The schema depicts a model of SK1/CK1 activation in response to IL-1β with downstream pathways leading to COX-2 induction/$cPLA_2$ activation, respectively. As both $cPLA_2$ and COX-2 are rate limiting for PGE_2 production, this model predicts and explains the synergistic formation of PGE_2 in response to the coordinate addition and/or generation of S1P and C1P.

production of the key inflammatory mediator PGE_2 in response to proinflammatory cytokines.

Overall, the results demonstrate that IL-1β activates SK1 and CK1 independent of each other and that these two enzymes independently regulate the induction of COX-2 and the translocation/activation of $cPLA_2$. In addition, we show that the products of each pathway, S1P and C1P, exert unique nonoverlapping actions on COX-2 and $cPLA_2$, respectively (Fig. 5). Furthermore, we demonstrate that these two sphingolipid products exhibit a significant synergistic response in the production of PGE_2 such that concentrations of S1P and C1P that had minimal individual effects on PGE_2 production induced potent PGE_2 production that matched and, at high doses, even exceeded the levels of PGE_2 normally induced by cytokines. Finally, we show that this coordinate and synergistic regulation reproduces activation of $cPLA_2$ and COX-2 in response to IL-1β even to the level of the pattern of protein colocalization as shown in confocal overlays.

Taken together, the data define a specific mechanism that operates to regulate the two key and rate-limiting enzymes in the production of the inflammatory mediator PGE_2 (Fig. 5). This mechanism ensures coordinate activation and translocation of $cPLA_2$ and induction of COX-2, leading to colocalization in space and time of arachidonate, the product of the first reaction and the substrate of the second COX-2 mediated reaction, which ultimately results in the formation of prostaglandins.

Much remains to be understood and discovered with regard to the mechanism of SK1 activation, S1P downstream signaling leading to COX-2 induction, and the mechanism of activation of CK1 upstream of C1P-medicated $cPLA_2$ translocation. However, the relative specificity and coordinate regulatory potency of these sphingolipid signaling pathways in response to cytokine stimulation already is sufficient to suggest that they may prove to be important pharmacologic targets for future development of novel anti-inflammatory and anticancer therapeutics.

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References

Baumruker T, Bornancin F, and Billich A (2005) The role of sphingosine and ceramide kinases in inflammatory responses. *Immunol Lett* **96:**175–185.

Billich A, Bornancin F, Mechtcheriakova D, Natt F, Huesken D, and Baumruker T (2005) Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1β and TNF-α induced production of inflammatory mediators. *Cell Signal*, in press.

Hinz B and Brune K (2002) Cyclooxygenase-2—10 years later. *J Pharmacol Exp Ther* **300:**367–375.

Hirabayashi T and Shimizu T (2000) Localization and regulation of cytosolic phospholipase A_2. *Biochim Biophys Acta* **1488:**124–138.

Li RC, Schentag JJ, and Nix DE (1993) The fractional maximal effect method: a new way to characterize the effect of antibiotic combinations and other nonlinear pharmacodynamic interactions. *Antimicrob Agents Chemother* **37:**523–532.

Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, and Davis RJ (1993) cPLA2 is phosphorylated and activated by MAP kinase. *Cell* **72:**269–278.

Ogretmen B and Hannun YA (2004) Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer* **4:**604–616.

Pettus BJ, Bielawska A, Spiegel S, Roddy P, Hannun YA, and Chalfant CE (2003a) Ceramide kinase mediates cytokine- and calcium ionophore-induced arachidonic acid release. *J Biol Chem* **278:**38206–38213.

Pettus BJ, Bielawska A, Subramanian P, Wijesinghe DS, Maceyka M, Leslie CC, Evans JH, Freiberg J, Roddy P, Hannun YA, et al. (2004) Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. *J Biol Chem* **279:** 11320–11326.

Pettus BJ, Bielawski J, Porcelli AM, Reames DL, Johnson KR, Morrow J, Chalfant CE, Obeid LM, and Hannun YA (2003b) The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. *FASEB J* **17:**1411–1421.

Pitson SM, Moretti PA, Zebol JR, Xia P, Gamble JR, Vadas MA, D'Andrea RJ, and Wattenberg BW (2000) Expression of a catalytically inactive sphingosine kinase mutant blocks agonist-induced sphingosine kinase activation. *J Biol Chem* **275:** 33945–33950.

Simmons DL, Botting RM, and Hla T (2004) Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacol Rev* **56:**387–437.

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