Compensatory Prostaglandin E_2 Biosynthesis in Cyclooxygenase 1 or 2 Null Cells

By Kanyawim Kirtikara,[‡] Scott G. Morham,[¶] Rajendra Raghow,[*‡‖] Stanley J. F. Laulederkind,[‡] Takuro Kanekura,[‡] Sarita Goorha,[*] and Leslie R. Ballou[*‡§]

From the [*]Department of Veterans Affairs Medical Center, the [‡]Department of Medicine, the [§]Department of Biochemistry, and the [‖]Department of Pharmacology, The University of Tennessee, Memphis, Tennessee 38163; and the [¶]Department of Pathology, The University of North Carolina, Chapel Hill, North Carolina 27559

Summary

Prostaglandin E_2 (PGE_2) production in immortalized, nontransformed cells derived from wild-type, cyclooxygenase 1–deficient ($COX\text{-}1^{-/-}$) or cyclooxygenase 2–deficient ($COX\text{-}2^{-/-}$) mice was examined after treatment with interleukin (IL)-1β, tumor necrosis factor α, acidic fibroblast growth factor, and phorbol ester (phorbol myristate acetate). Compared with their wild-type counterparts, $COX\text{-}1^{-/-}$ or $COX\text{-}2^{-/-}$ cells exhibited substantially enhanced expression of the remaining functional COX gene. Furthermore, both basal and IL-1–induced expression of cytosolic phospholipase A_2 ($cPLA_2$), a key enzyme-regulating substrate mobilization for PGE_2 biosynthesis, was also more pronounced in both $COX\text{-}1^{-/-}$ and $COX\text{-}2^{-/-}$ cells. Thus, $COX\text{-}1^{-/-}$ and $COX\text{-}2^{-/-}$ cells have the ability to coordinate the upregulation of the alternate COX isozyme as well as $cPLA_2$ genes to overcome defects in prostaglandin biosynthetic machinery. The potential for cells to alter and thereby compensate for defects in the expression of specific genes such as COX has significant clinical implications given the central role of COX in a variety of disease processes and the widespread use of COX inhibitors as therapeutic agents.

Prostaglandins, such as prostaglandin (PG)E_2,[1] are pivotal modulators of tissue homeostatsis and their aberrant regulation is known to cause serious pathophysiological consequences (1–4). PGE_2 biosynthesis is regulated by successive metabolic steps involving the phospholipase A_2–mediated release of arachidonic acid (AA) and its conversion to PGE_2 by cyclooxygenase (COX), hydroperoxidase, and isomerase activities (1–4). Although cytosolic phospholipase A_2 ($cPLA_2$) is primarily responsible for agonist-induced AA release from membrane phospholipids (5, 6), secretory PLA_2 may also be important in regulating AA availability via a transcellular mechanism (7). Conversion of AA to PGH_2, the commited step in prostanoid biosynthesis, is mediated by cyclooxygenases, COX-1 and -2, which are encoded by two unique genes, located on different chromosomes (2). Generally, although COX-1 is constitutively expressed, the expression of COX-2 is highly inducible (2, 3). Based on their respective modes of expression, it is thought that COX-1 is primarily involved in cellular homeostasis, whereas COX-2 plays a major role in inflammation and mitogenesis. The COX isoenzymes are thought to be the primary target enzymes for nonsteroidal antiinflammatory drugs (NSAIDs), which act by inhibiting the COX activity of COX-1 and -2, thereby blocking their ability to convert AA to PGG_2 (8, 9). In addition to the use of nonsteroidal antiinflammatory drugs as analgesics and for alleviation of acute and chronic inflammation, these agents have proven effective in decreasing the frequency of heart attacks and strokes (8, 10, 11), and in reducing the incidence of colon cancer (12, 13).

Since most cells invariably express both COX-1 and -2 under the appropriate conditions, it has been somewhat problematic to determine the exact contribution of the two COX isozymes towards basal and agonist-inducible PGE_2 biosynthesis; the use of selective COX-1/COX-2 inhibitors to define relative contributions of the two isozymes has also resulted in limited success. The purpose of this study was to examine the effects of COX deficiency on the differential expression of the COX-1 and -2 isozymes, and compare the responses of wild-type, COX-1, and COX-2 knockout cells with respect to agonist-induced PGE_2 bio-

[1] *Abbreviations used in this paper:* AA, arachidonic acid; COX, cyclooxygenase; cPLA, cytosolic phospholipase; FGF, fibroblast growth factor; PG, prostaglandin; RIA, radioimmunoassay.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/98/02/517/07 $2.00
Volume 187, Number 4, February 16, 1998 517–523
http://www.jem.org

synthesis. We demonstrate that the expression of COX, $cPLA_2$, and PGE_2 production are significantly increased in COX-deficient cells. Thus, COX deficiency, regardless of whether it is COX-1 or -2, results in the enhanced basal and inducible expression of the remaining COX isozyme as well as the elevated expression of $cPLA_2$. We interpret these data to indicate that the elevated production of PGE_2 in COX-1 or -2 isozyme-deficient cells is due to the compensatory expression of the remaining COX isozyme.

Materials and Methods

Isolation and Culture of COX-deficient Mouse Cells. Lungs were collected from wild-type C57BL/6J (B6), COX-1–deficient (14), and COX-2–deficient (15) mice. The tissues were dissected into small pieces and grown underneath coverslips in 10-cm plates with MEM supplemented with PenStrep at a concentration of 300,000 U/liter penicillin G and 300 mg/liter streptomycin sulfate, nonessential amino acids (0.1 mM), Fungizone (1 mg/liter Amphotericin B), glutamine (292 mg/liter), ascorbic acid (50 mg/liter), and 10% FCS in a humidified incubator with 5% CO_2, and the media were changed three times per week. After 3 wk of culture under these conditions, only fibroblasts continued to grow. The PenStrep was reduced to 100,000 U/liter penicillin G and 100 mg/liter streptomycin sulfate and the media were replaced twice per week for another 3–5 wk. During subsequent passages, cells were maintained in DMEM containing high glucose and supplemented with PenStrep (100,000 U/liter penicillin G and 100 mg/liter streptomycin sulfate), nonessential amino acids (0.1 mM), Fungizone (1 mg/liter Amphotericin B), glutamine (292 mg/liter), ascorbic acid (50 mg/liter), and 10% FCS.

Transfection/Immortalization of COX-deficient Cells. Subconfluent monolayers of COX-$1^{-/-}$ or COX-$2^{-/-}$ lung fibroblasts (passages 5–7) were cotransfected with pLE12S (containing adenovirus E1A gene; 8 μg/10-cm diam dish) and pREP4 (containing a hygromycin resistance gene; Invitrogen, Carlsbad, CA) plasmids (2 μg/10-cm diam dish) by the LipofectAMINE reagent (GIBCO BRL, Gaithersburg, MD). Plasmid pLE12S was a gift of Dr. Margaret Quinlan (University of Tennessee, Memphis, TN). Cells were maintained in the above media containing hygromycin (50 μg/ml) for 1 wk; hygromycin in the culture media was increased 50 μg/ml per week until the final concentration reached 250 μg/ml. Subsequent cell passage and subculture of cells used in all experiments was done in media containing 250 μg/ml hygromycin.

Treatment of COX-$1^{-/-}$ and COX-$2^{-/-}$ Cells with Cytokines and PMA. Cells were seeded at 10^5 cells/ml in DMEM (high glucose) supplemented with PenStrep (100,000 U/liter penicillin G and 100 mg/liter streptomycin sulfate), nonessential amino acids (0.1 mM), Fungizone (1 mg/liter Amphotericin B), glutamine (292 mg/liter), ascorbic acid (50 mg/liter), 10% FCS, and 250 μg/ml hygromycin in 24-well (0.9 ml/well) flat-bottomed tissue culture plates (Costar, Cambridge, MA). Cells were incubated at 37°C in a humidified CO_2 incubator (5% CO_2) for 48 h until confluent. The medium was then replaced with fresh DMEM containing 0.5% FCS. Where indicated, cells were treated with IL-1 (0.25 ng/ml), TNF (5 ng/ml), acidic fibroblast growth factor (FGF; 10 ng/ml), or PMA (12.5 ng/ml) along with the appropriate vehicle controls; at these concentrations, neither cytokines nor PMA affected cell morphology or viability. In Western blot experiments, cells were treated as above except they were seeded in 6-well culture plates (2.7×10^5 cells/well).

Western Blot Analysis. The medium from COX-deficient cells cultured in 6-well plates was aspirated and cell monolayers were washed with cold PBS and lysed in the Laemmli sample buffer. The samples were boiled for 3 min, and identical amounts of protein applied to SDS-PAGE (7.5%) and later transblotted to an Optitran membrane (Schleicher & Schuell, Keene, NH). The blot was blocked with 5% nonfat dry milk before incubation with either a rabbit antibody against murine COX-2 (Cayman Chemical, Ann Arbor, MI), a rabbit antibody against murine COX-1 (provided by Dr. D. DeWitt; reference 16), or mouse monoclonal anti-$cPLA_2$ antiserum (Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were detected using either enhanced chemiluminescence or enhanced chemifluorescence kits from Amersham Corp. (Arlington Heights, IL).

PGE_2 Measurement. PGE_2 in the media was measured by radioimmunoassay (RIA); this assay is based upon the competition by PGE_2 in the test sample with labeled PGE_2 for anti-PGE_2 antibody binding sites. A 2–100-μl aliquot of culture medium was added to RIA assay buffer (0.1 mM phosphate buffer, pH 7.4, containing 0.9% sodium chloride, 0.1% sodium azide, and 0.1% gelatin), and mixed with the appropriate amount of labeled tracer and reconstituted antiserum. The mixture was incubated overnight at 4°C. Assay tubes were then placed in an ice bath, and 1 ml of cold charcoal-dextran suspension was added. After a 15-min incubation, the tubes were centrifuged at 2,200 *g* for 10 min at 4°C; the supernatants were decanted into scintillation vials, and radioactivity was determined by scintillation spectrometry. Percent binding was compared against a standard curve, and the amount of PGE_2 in the sample was calculated. In each case the amount of PGE_2 produced was normalized by cell number and all data is presented as picograms of PGE_2 per 10^3 cells. To determine the potential effect of nonenzymatically produced, PGE_2-immunoreactive products (e.g., isoprostanes) on RIA results, assays were performed in the presence of an effective COX-1 inhibitor (indomethacin, 1 mM) and NS-398 (1 μM), a COX-2 inhibitor, both of which block PGE_2 biosynthesis, but should not block the production of nonenzymatically generated AA metabolites; products were further analyzed by radio-thin-layer chromatography (17).

Statistical Analysis. Paired *t* test was used to determine the differences in the PGE_2 levels between control samples of wild-type, COX-$1^{-/-}$ and COX-$2^{-/-}$ cells, and between control samples and samples from cytokine-treated cells. Differences were considered significant if $P < 0.05$.

Results and Discussion

We examined the effects of IL-1 on PGE_2 production in cells containing both COX isozymes (wild type) compared with cells that had only COX-1 (COX-$2^{-/-}$) or COX-2 (COX-$1^{-/-}$), respectively. As shown in Fig. 1 *A* both COX-$1^{-/-}$ or COX-$2^{-/-}$ cells synthesized 6–8-fold higher amounts of PGE_2 compared with their wild-type counterparts. Interestingly, basal PGE_2 production was higher in both COX-$2^{-/-}$ (66.71 ± 3.54 pg/10^3 cells; $n = 6$) and COX-$1^{-/-}$ (90.23 ± 3.29 pg/10^3 cells; $n = 8$) cells compared with wild-type (11.07 ± 0.62 pg/10^3 cells; $n = 8$). (All values are mean ± SE.) IL-1 treatment of wild-type and COX-$1^{-/-}$ cells further enhanced their PGE_2 output. In contrast, IL-1 treatment of COX-$2^{-/-}$ cells did not significantly enhance PGE_2 biosynthesis (Fig. 1 *A*).

These dramatic differences in basal PGE_2 biosynthesis between wild-type and COX-deficient cells, prompted us to compare the expression of genes encoding three key en-

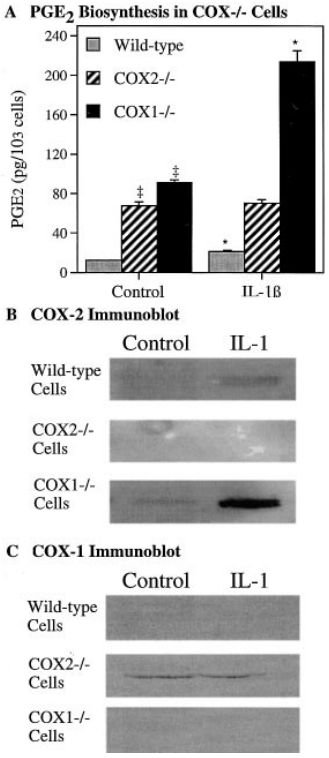

Figure 1. The effect of IL-1 on PGE_2 biosynthesis, COX-1, and -2 expression. (*A*) Wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells were treated with vehicle (*Control*) or with IL-1 (0.25 ng/ml) as described in Materials and Methods. After 24 h of culture, media was collected and analyzed for PGE_2 by RIA. Data are means ± SE of at least six separate determinations (wells). ‡ Values that are significantly different from the wild-type value in the control group by paired *t* test ($P < 0.05$). * Values that are significantly different from the control values of each respective cell type by paired *t* test. (*B*) Western blot analysis of COX-2 protein levels in IL-1–stimulated cells. Wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells were treated with vehicle (*Control*) or with IL-1 (0.25 ng/ml) as described in Materials and Methods. After 24 h of culture, media was removed and Western blot analysis was carried out as described in Materials and Methods. (*C*) Western blot analysis of COX-1 protein levels in IL-1–stimulated cells. Wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells were treated with vehicle (*Control*) or with IL-1 (0.25 ng/ml) as described in Materials and Methods. After 24 h of culture, media was removed and Western blot analysis was carried out as described in Materials and Methods.

zymes (COX-1, COX-2, and $cPLA_2$) regulating PGE_2 biosynthesis in untreated and IL-1–treated wild-type, COX-$1^{-/-}$, and COX-$2^{-/-}$ cells. A comparison of basal and IL-1–stimulated levels of COX-1 and COX-2 protein by immunoblot assay in wild-type and COX-$1^{-/-}$ cells is shown in Fig. 1, *B* and *C*. Consistent with numerous previous observations, the basal expression of COX-2 protein in wild-type cells was barely detectable (Fig. 1 *B*). Constitutive levels of COX-2 proteins were also significantly increased (2.4-fold) in untreated COX-$1^{-/-}$ cells (Fig. 1 *B*). The elevated level of COX-2 protein correlates well with the higher basal PGE_2 levels in COX-$1^{-/-}$ cells compared with those in wild-type cells. When treated with IL-1, COX-2 protein levels increased moderately in wild-type cells (Fig. 1 *B*), but the increase in COX-2 protein was much more dramatic in COX-$1^{-/-}$ cells (41-fold). The overall pattern of COX-2 protein expression in wild-type and COX-$1^{-/-}$ cells correlated with increased PGE_2 production seen in cells with unique COX phenotypes (see Fig. 1 *A*).

Next, we examined the basal and IL-1–stimulated levels of COX-1 protein in identically treated wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells, respectively (Fig. 1 *C*). In wild-type cell extracts, the level of COX-1 protein was barely detectable and IL-1 treatment was apparently inconsequential. This result was not unexpected since COX-1 expression is not known to be inducible under many conditions. We observed that basal expression of COX-1 protein in untreated COX-$2^{-/-}$ cells (Fig. 1 *C*) was much greater (14-fold) than that in wild-type cells. This overexpression of COX-1 protein corresponds with greater basal PGE_2 levels in COX-$2^{-/-}$ cells, compared with the basal levels in wild-type cells. IL-1 had no stimulatory effect on COX-1 protein levels in COX-$2^{-/-}$ cells (Fig. 1 *C*) and as expected, COX-$1^{-/-}$ cells did not express detectable COX-1 protein. Another important enzyme in the prostaglandin biosynthetic pathway is PGE_2 synthase, the isomerase that converts PGH_2 to PGE_2. Although PGE_2 synthase has neither been sequenced nor cloned, making it difficult to study, available evidence does seem to indicate that this enzyme is not a rate-limiting reaction in PGE_2 biosynthesis. However, based upon our findings, we cannot rule out the possibility that PGE_2 synthase expression may also be altered in COX null cells.

To examine the possibility that iso-PGE_2 or other isoprostanes (18) may be generated nonenzymatically from a buildup of endoperoxide intermediate that cross-reacts with the anti-PGE_2 used in our RIA leading to erroneously high estimations of COX and/or PGE_2 synthase activity, we performed two experiments. First, we treated wild-type and COX$^{-/-}$ cells with either indomethacin or NS-398, COX-1, and COX-2 selective inhibitors, respectively since these COX inhibitors should block PGE_2 synthesis without affecting iso-PGE_2 formation. We found that either indomethacin or NS-398 completely blocked both the basal and cytokine-induced formation of immunoreactive PGE_2 in wild-type and COX$^{-/-}$ cells (data not shown). Second, radio-thin-layer chromatography was used to confirm that PGE_2 was by far the predominate prostanoid product generated by wild-type and COX$^{-/-}$ cells and that no other AA metabolites in addition to PGE_2 were generated in COX$^{-/-}$ cells (data not shown).

To compare the effects of IL-1 (see Fig. 1 *A*) to other inducers of PGE_2 biosynthesis, we tested the effects of TNF, acidic FGF, and PMA on PGE_2 production in wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells (Fig. 2). Compared to stimulated wild-type cells, there was significantly more PGE_2 produced in either COX-$1^{-/-}$ or COX-$2^{-/-}$ cells, with the possible exception of TNF that induced comparable PGE_2 biosynthesis in each cell type. In response to FGF, the amount of PGE_2 that was produced by COX-$2^{-/-}$ cells was elevated and in COX-$1^{-/-}$ cells, PGE_2 was even more dramatically elevated compared to wild type. Both COX-$2^{-/-}$ and COX-$1^{-/-}$ cells treated with PMA also produced much more PGE_2 than wild type. Thus, in general, COX-isozyme deficiency results in increased PGE_2 biosynthesis, but the relative contributions of COX-1 and COX-2 are clearly dependent upon the specific agonists involved.

Since constitutive COX-2 protein expression and PGE_2 production in COX-$1^{-/-}$ cells was significantly enhanced, we were also curious about the status of $cPLA_2$ gene expression in COX-deficient cells. We reasoned that $cPLA_2$ activity could be involved in regulating levels of free AA for conversion to PGE_2 and thereby could play a critical role in compensating for COX-isozyme deficiency. We were

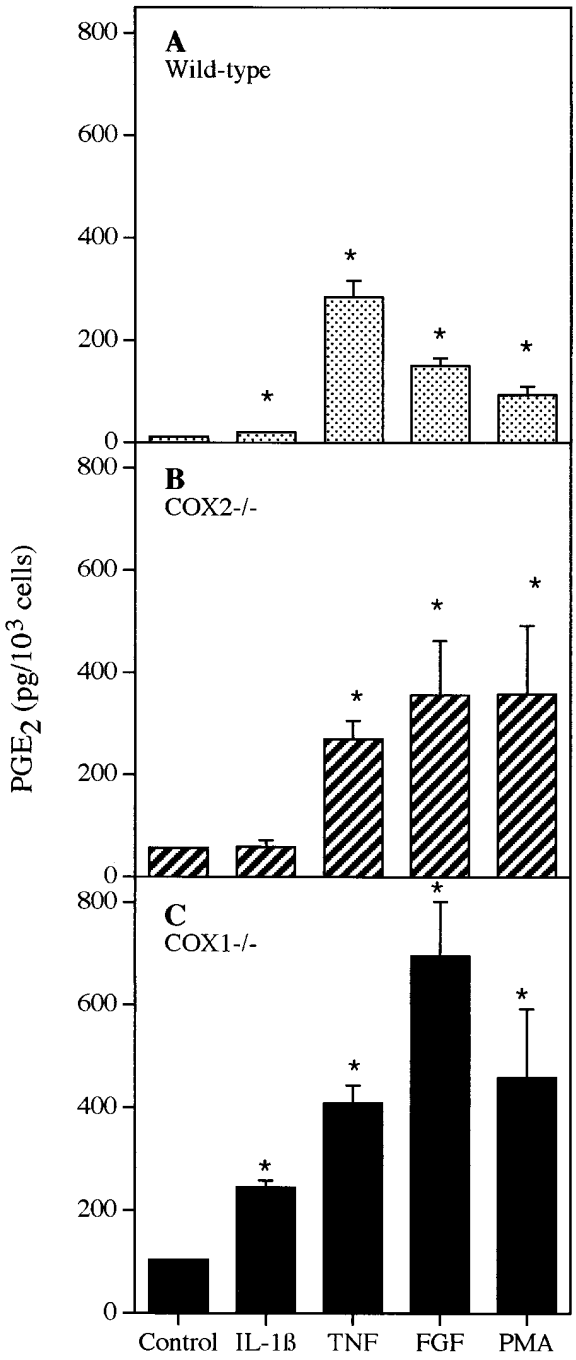

Figure 2. The effect of cytokines and PMA on PGE_2 biosynthesis. Wild-type (*A*), COX-$2^{-/-}$ (*B*), and COX-$1^{-/-}$ (*C*) mouse cells were treated with IL-1 (0.25 ng/ml), TNF (5 ng/ml), FGF (10 ng/ml), or PMA (12.5 ng/ml) as described in Materials and Methods. After 24 h of culture, media was collected and analyzed for PGE_2 by RIA. Data are means ± SE of at least six separate determinations (wells). * Values that are significantly different from the control values of each respective cell type by paired *t* test ($P < 0.05$).

somewhat surprised to find that basal levels of $cPLA_2$ protein in either COX-$2^{-/-}$ (Fig. 3 *B*) or COX-$1^{-/-}$ (Fig. 3 *C*) cells were significantly higher than levels of $cPLA_2$ in wild-type cells (Fig. 3 *A*). It is conceivable, therefore, that enhanced expression of $cPLA_2$ could directly contribute to higher PGE_2 levels in both of the COX-deficient cells by generating greater AA substrate for PGE_2 biosynthesis. Treatment of COX-$1^{-/-}$ or COX-$2^{-/-}$ cells with IL-1 resulted in a modest increase in the amount of $cPLA_2$ protein (4-fold in COX-$1^{-/-}$ and 1.4-fold in COX-$2^{-/-}$). This was in contrast to wild-type cells, which showed no change in the levels of $cPLA_2$ protein after treatment with IL-1. As an important control, we examined the quantitative parameters of PGE_2 production, and COX-1, COX-2, and $cPLA_2$ gene expression in wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ cells from primary cell cultures and found essentially the same patterns in primary cells as those observed in the immortalized cells (data not shown). Therefore, the characteristic pattern of expression of COX-1, COX-2, and $cPLA_2$ proteins in COX-$2^{-/-}$ and COX-$1^{-/-}$ cells is not elicited as a result of immortalization caused by the E1A adenovirus gene. Taken together these data indicate that COX-$1^{-/-}$ cells express enhanced levels of both basal and cytokine-stimulated COX-2 protein, and increased basal expression of $cPLA_2$ protein. We postulate that the significantly increased levels of COX-2 and $cPLA_2$ in COX-$1^{-/-}$ cells are likely to account for the increased rates of PGE_2 biosynthesis; these data also implicate the existence of compensatory mechanisms for PGE_2 production in COX-isozyme–deficient cells.

To distinguish between preferences of COX-1 and -2 for endogenous and/or exogenous AA for conversion to PGE_2, and to verify that COX-1 was indeed expressed in COX-$2^{-/-}$ cells as judged by its ability to synthesize PGE_2, we added exogenous AA to wild-type, COX-$2^{-/-}$, or COX-$1^{-/-}$ cells that were either untreated or treated with IL-1, TNF, FGF, or PMA. The results shown in Fig. 4 demonstrate that cells expressing only COX-1 (COX-$2^{-/-}$) synthesized similar amounts of PGE_2 as wild-type or COX-$1^{-/-}$ cells supplemented with exogenous AA. Therefore, COX-1 is expressed and enzymatically active in COX-$2^{-/-}$ cells, but cytokines neither enhance COX-1 protein biosynthesis nor PGE_2 biosynthesis. Thus, agonists that induce PGE_2 biosynthesis in COX-$2^{-/-}$ cells in the absence of exogenous AA, do so by increasing endogenous substrate availability. Based on these data, we conclude that

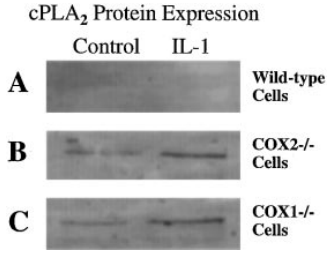

Figure 3. Expression of $cPLA_2$ in IL-1–stimulated cells. Wild-type, COX-$2^{-/-}$, and COX-$1^{-/-}$ mouse cells were treated with vehicle (*Control*) or with IL-1 (0.25 ng/ml) as described in Materials and Methods. After 24 h of culture, media was removed and Western blot analysis was carried out as described in Materials and Methods. (*A*) Proteins from wild-type cells. (*B*) Proteins from COX-$2^{-/-}$ cells. (*C*) Proteins from COX-$1^{-/-}$ cells. *A*, *B*, and *C* are from the same blot, which is a representative of three replications.

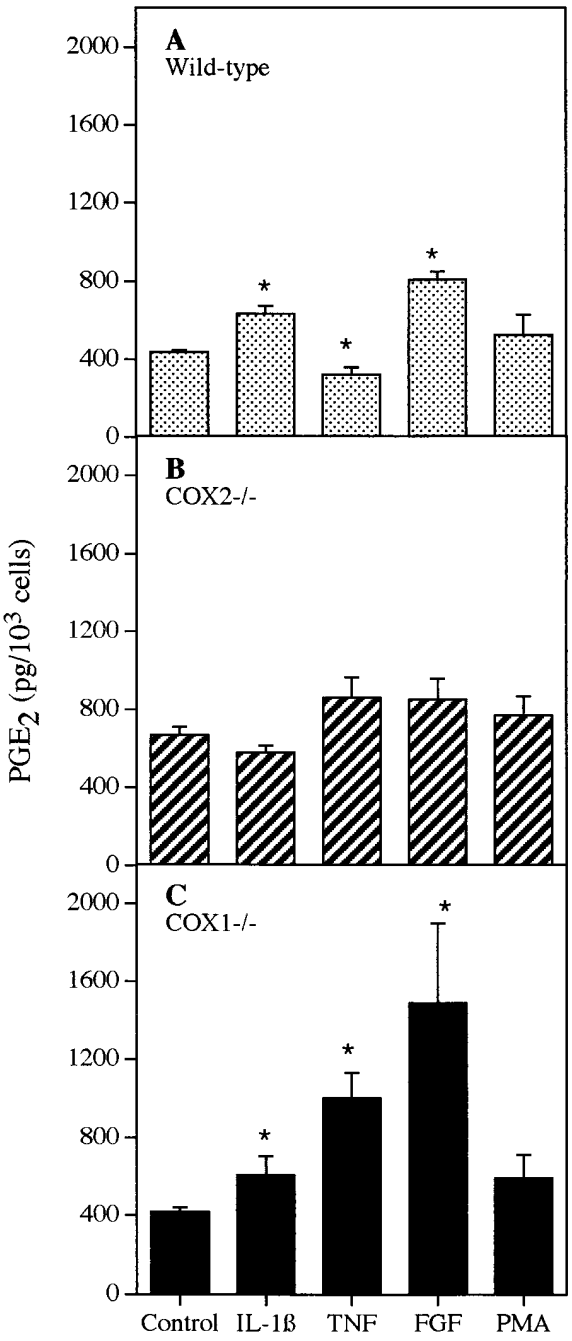

Figure 4. The effect of exogenous arachidonic acid on cytokines or PMA induced PGE_2 production. Wild-type (*A*), COX-$2^{-/-}$ (*B*), and COX-$1^{-/-}$ (*C*) mouse cells were treated with IL-1 (0.25 ng/ml), TNF (5 ng/ml), FGF (10 ng/ml), or PMA (12.5 ng/ml) as described in Materials and Methods. After 24 h of culture, media was removed, the cells were washed to remove any accumulated PGE_2, and fresh serum-free media containing AA (50 μM) was added. After a 15-min incubation in the presence of added AA, media was collected and PGE_2 measured by RIA. Data are means ± SE of at least six separate determinations (wells). * Values that are significantly different from the control values of each respective cell type by paired *t* test ($P < 0.05$).

in COX-$2^{-/-}$ cells, substrate is likely to be limiting for constitutively expressed COX-1–mediated PGE_2 biosynthesis. Fig. 4 also shows that COX-$1^{-/-}$ cells are able to use both exogenous and endogenous substrates (also see Fig. 1 *A*). However, IL-1, TNF, and FGF significantly enhanced the ability of COX-$1^{-/-}$ cells to produce PGE_2, most likely by enhancing COX-2 expression as shown in Fig. 2. In addition, COX-$1^{-/-}$ cells treated with PMA did not produce elevated levels of PGE_2, even when exogenous AA was provided. This indicates that PMA likely increased PGE_2 production by increasing the availability of endogenous AA in COX-$1^{-/-}$ cells, whereas IL-1, TNF, and FGF likely affect AA mobilization and COX-2 expression. PMA affected the wild-type cells similarly. These results clearly raise the possibility that in COX-1 or -2 null cells, there is a coordinate upregulation of the expression and/or activities of COX-1, COX-2, and $cPLA_2$, leading to increased PGE_2 biosynthesis. These data also demonstrate that both COX-$1^{-/-}$ and COX-$2^{-/-}$ cells can effectively use AAs from either endogenous or exogenous sources.

Our data are consistent with the hypothesis that the long-term of COX-isozyme deficiency results in the altered expression of the remaining two enzymes that regulate mobilization and conversion of arachidonic acid to prostaglandins. Fig. 5 summarizes the patterns of COX and $cPLA_2$ expression in COX null cells compared with normal cells in response to IL-1. The scheme shows the compensatory expression of the alternative COX isozyme and $cPLA_2$ when one of the COX isozymes is absent. In cells lacking the housekeeping isozyme COX-1, overcompensation results in the overexpression of COX-2 and $cPLA_2$, and in turn elevated PG biosynthesis. Similarly, cells lacking the inducible isozyme, COX-2, elicit the enhanced expression of COX-1 and $cPLA_2$. Although we are uanble to comment as to the precise status of PGE_2 synthase expression in wild-type, COX-$1^{-/-}$ or COX-$2^{-/-}$ cells, we have depicted its expression in each cell type; since PGE_2 is the predominate prostanoid product, its expression would not appear to be rate-limiting given the great potential for PGE_2 biosynthesis in the presence of exogenous AAs (see Fig. 4). Thus, our data clearly show that COX-deficient cells have the potential to overcome the lack of expression of one or the other COX isoenzymes by overexpressing the alternate COX isoform and increased $cPLA_2$ expression. Such a potential mechanism for producing PGE_2 by cells in vitro is not surprising since neither COX-$1^{-/-}$ (14) nor COX-$2^{-/-}$ mice (15) showed severe developmental arrest in utero or immediate postnatal mortality. However, in contrast to the results shown here using lung fibroblasts, Langenbach et al. (14) did not report any compensatory COX-2–mediated PGE_2 production in glandular stomachs of COX-1–deficient mice, suggesting that tissue specificity may also be an important factor for further investigation. Together, these findings underscore the importance of elucidating the potential long-term effects of COX-1 or COX-2 inhibition with respect to alterations in the quantitiative and/or qualitative patterns of AA metabolism.

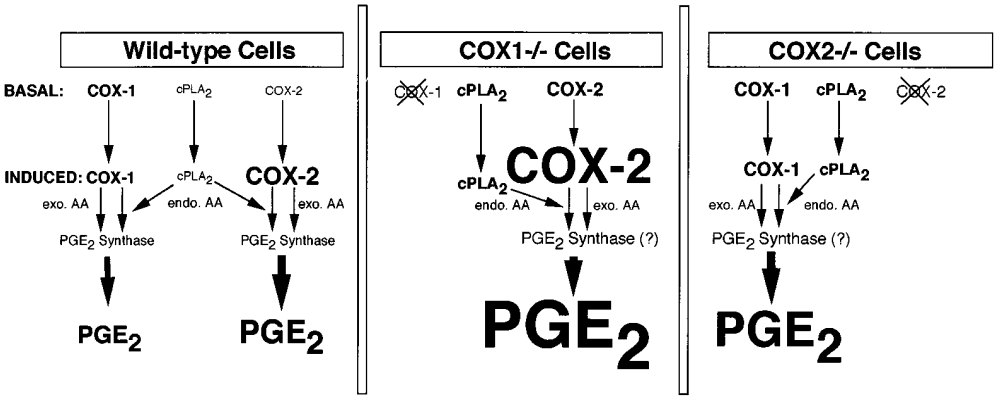

Figure 5. The coordinate regulation of COX-1, COX-2, and $cPLA_2$ expression in COX null cells by IL-1. In wild-type cells, inducible COX-2 is responsible for the increase in IL-1–induced PGE_2 production as represented by proportionally larger characters and lines throughout the diagram. Although the constitutive level of COX-1 expression is low, exogenous AA (*exo. AA*) is effectively converted to PGE_2. In COX-$2^{-/-}$ or COX-$1^{-/-}$ cells, the overexpression of $cPLA_2$ may play a role in increased basal PGE_2 biosynthesis compared to wild-type cells by increasing the availability of endogenous AA (*endo. AA*). IL-1 greatly induces COX-2 accumulation in COX-$1^{-/-}$ cells resulting in enhanced PGE_2 biosynthesis. In COX-$2^{-/-}$ cells, overexpression of COX-1 and $cPLA_2$ lead to an increase in basal PGE_2 biosynthesis compared to wild type. However, IL-1 does not enhance PGE_2 biosynthesis in COX-$2^{-/-}$ due to the lack of increased COX-1 expression. As in wild-type cells, exogenous AA is effectively used by COX-1 in COX-$2^{-/-}$ cells as indicated by a high level of PGE_2 accumulation. At present, the effects of COX-isozyme deficiency on the expression of PGE_2 synthase are not known, but this enzyme does not appear to be rate limiting.

This work was supported by research funds from the Department of Veterans Affairs (DVA), The Arthritis Foundation, and grants AR39166 and AR26034 from the National Institutes of Health (National Institute of Arthritis and Musculoskeletal and Skin Diseases). R. Raghow is a Career Scientist of the DVA.

Address correspondence to Leslie R. Ballou, Department of Veterans Affairs Medical Center, Research Service (151), 1030 Jefferson Avenue, Memphis, TN 38104. Phone: 901-577-7283. Fax: 901-577-7273; E-mail: lballou@utmem1.utmem.edu

Received for publication 17 September 1997 and in revised form 18 November 1997.

References

1. Goetzl, E.J., S. An, and W.L. Smith. 1995. Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB J.* 9:1051–1058.
2. Smith, W.L., R.M. Garavito, and D.L. DeWitt. 1996. Prostaglandin endoperoxide H synthase (cyclooxygenases)-1 and -2. *J. Biol. Chem.* 271:33157–33160.
3. Herschman, H. 1996. Prostaglandin synthase 2. *Biochim. Biophys. Acta.* 1299:125–140.
4. Herschman, H.R., R.S. Gilbert, W. Xie, S. Luner, and S.T. Reddy. 1995. The regulation and role of TIS10 prostaglandin synthase-2. *Adv. Prostaglandin Thromboxane Leukotreine Res.* 23:23–28.
5. Lin, L.L., A.Y. Lin, and J.L. Knopf. 1992. Cytosolic phospholipase A_2 is coupled to hormonally regulated release of arachidonic acid. *Proc. Natl. Acad. Sci. USA.* 89:6147–6151.
6. Clark, J.D., L.L. Lin, R.W. Kriz, C.S. Ramesha, L.A. Sultzman, A.Y. Lin, N. Milona, and J.L. Knopf. 1991. A novel arachidonic acid-selective cytosolic PLA_2 contains a $Ca^{(2+)}$-dependent translocation domain with homology to PKC and GAP. *Cell.* 65:1043–1051.
7. Reddy, S.T., and H.R. Herschman. 1996. Transcellular prostaglandin production following mast cell activation is mediated by proximal secretory phospholipase A_2 and distal prostaglandin synthase 1. *J. Biol. Chem.* 271:186–191.
8. Vane, J.R., and R.M. Botting. 1995. A better understanding of anti-inflammatory drugs based on isoforms of cyclooxygenase (COX-1 and COX-2). *Adv. Prostaglandin Thromboxane Leukotreine Res.* 23:41–48.
9. DeWitt, D.L., E.A. Meade, and W.L. Smith. 1993. PGH synthase isozyme selectivity: potential safer nonsteroidal anti-inflammatory drugs. *Am. J. Med.* 95:40s–44s.
10. Vane, J.R., and R.M. Botting. 1994. Regulatory mechanisms of the vascular endothelium: an update. *Pol. J. Pharmacol.* 46: 499–421.
11. Vane, J.R., and R.M. Botting. 1995. New insights into the mode of action of anti-inflammatory drugs. *Inflamm. Res.* 44: 1–10.
12. Kargman, S.L., G.P. O'Neill, P.J. Vickers, J.F. Evans, J.A. Mancini, and S. Jothy. 1995. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. *Cancer Res.* 55:2556–2559.
13. Rigas, B., I.S. Goldman, and L. Levine. 1993. Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.* 122: 518–523.
14. Langenbach, R., S.G. Morham, H.F. Tiano, C.D. Loftin, B.I. Ghanayem, P.C. Chulada, J.F. Mahler, C.A. Lee, E.H. Goulding, K.D. Kluckman, and O. Smithies. 1995. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell.* 83:483–492.

15. Morham, S.G., R. Langenbach, C.D. Loftin, H.F. Tiano, N. Vouloumanos, J.C. Jennette, J.F. Mahler, K.D. Kluckman, A. Ledford, C.A. Lee, and O. Smithies. 1995. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell*. 83:473–482.
16. Morita, I., M. Schindler, M.K. Regier, J.C. Otto, T. Hori, D.L. De Witt, and W.L. Smith. 1995. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J. Biol. Chem*. 270:10902–10908.
17. Baily, J.M., B. Muza, T. Hla, and K. Salata. 1985. Restoration of prostacyclin synthase in vascular smooth muscle cells after aspirin treatment: regulation by epidermal growth factor. *J. Lipid Res*. 26:54–61.
18. Roberts, L.J., and J.D. Morrow. 1997. The generation and actions of isoprostanes. *Biochim. Biophys. Acta*. 1345:121–135.