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Human cyclooxygenase-2 cDNA

(prostaglandins/gene expression/angiogenesis/inflammation)

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ABSTRACT **Cyclooxygenase (Cox), also known as prostaglandin (PG) H synthase (EC 1.14.99.1), catalyzes the rate-limiting step in the formation of inflammatory PGs. A major regulatory step in PG biosynthesis is at the level of Cox: growth factors, cytokines, and tumor promoters induce Cox activity. We have cloned the second form of the Cox gene (Cox-2) from human umbilical vein endothelial cells (HUVEC). The cDNA encodes a polypeptide of 604 amino acids that is 61% identical to the previously isolated human Cox-1 polypeptide. *In vitro* translation of the human (h)Cox-2 transcript in rabbit reticulocyte lysates resulted in the synthesis of a 70-kDa protein that is immunoprecipitated by antiserum to ovine Cox. Expression of the hCox-2 open reading frame in Cos-7 monkey kidney cells results in the elaboration of cyclooxygenase activity. hCox-2 cDNA hybridizes to a 4.5-kilobase mRNA species in HUVEC, whereas the hCox-1 cDNA hybridizes to 3- and 5.3-kilobase species. Both Cox-1 and Cox-2 mRNAs are expressed in HUVEC, vascular smooth muscle cells, monocytes, and fibroblasts. Cox-2 mRNA was preferentially induced by phorbol 12-myristate 13-acetate and lipopolysaccharide in human endothelial cells and monocytes. Together, these data demonstrate that the Cox enzyme is encoded by at least two genes that are expressed and differentially regulated in a variety of cell types. High-level induction of the hCox-2 transcript in mesenchymal-derived inflammatory cells suggests a role in inflammatory conditions.**

A major mechanism for the control of prostaglandin (PG) synthesis occurs at the level of cyclooxygenase [Cox; also known as PGH synthase (EC 1.14.99.1)] (1, 2). Growth factors, cytokines, and tumor promoters appear to modulate PG synthesis by inducing the Cox enzyme. For example, epidermal growth factor induces Cox in vascular smooth muscle cells (3); interleukin 1 (IL-1) induces it in vascular endothelial cells, smooth muscle cells (4), and dermal fibroblasts (5); and platelet-derived growth factor, serum, and phorbol 12-myristate 13-acetate (PMA) induce it in murine 3T3 cells (6, 7), among others. Raz *et al.* (8), using a radioimmunoprecipitation assay, demonstrated that IL-1 induces the *de novo* synthesis of Cox protein. Recent studies have shown that anti-inflammatory steroids, such as dexamethasone (Dex), inhibit the IL-1 and lipopolysaccharide (LPS)-stimulated synthesis of Cox protein *in vitro* (9) and *in vivo* (10). Thus, the Cox enzyme appears to be a major regulatory step at which interactions between pro- and anti-inflammatory mediators occur.

The cellular and molecular mechanisms responsible for Cox enzyme regulation have been under intense investigation. Using the ovine Cox cDNA as a probe, Lin *et al.* (11) demonstrated that platelet-derived growth factor, PGE_2, and PMA induced the 3-kilobase (kb) mRNA for Cox in 3T3 cells. However, the kinetics of Cox mRNA induction did not correlate with the increase in PGE_2 synthesis. In human umbilical vein endothelial cells (HUVEC), we demonstrated that IL-1, an inhibitor of endothelial growth, induces the 3-kb Cox mRNA in a time- and dose-dependent manner (12). In contrast, heparin-binding growth factor 1 (HBGF-1), a potent mitogen for HUVEC, suppresses the Cox mRNA levels (13).

Recently, several cDNAs that are homologous to the Cox gene were cloned. Xie *et al.* (14) reported the cloning of a *src*-inducible cDNA, termed CEF-147; the 70-kDa deduced polypeptide encoded by the CEF-147 cDNA is homologous (59% identity) to the ovine, murine, and human Cox-1 sequences. In addition, Herschman and colleagues (15) reported the characterization of a 4.5-kb PMA-inducible immediate-early transcript, termed TIS10, from 3T3 cells. The murine TIS10 polypeptide also possessed significant (59%) sequence identity to the known Cox-1 sequences but was more closely related (82% identity) to the chicken Cox-2 (CEF147) polypeptide. While the deduced polypeptide sequences of these cDNAs contain amino acid residues essential for Cox enzyme activity, expression of these cDNAs into enzymatically active Cox protein has not been reported. Furthermore, the expression of the TIS10 mRNA was detected only in 3T3 and rat-1 cells (15).

In this communication, we describe the cloning, sequencing, and expression of human Cox-2 (hCox-2) cDNA from endothelial cells.† We also demonstrate that both hCox-1 and hCox-2 genes are expressed by a variety of normal cell types and are regulated differentially.

EXPERIMENTAL PROCEDURES

Cell Culture. HUVEC (passage 4–8) were grown on fibronectin-coated plastic dishes in media containing 10% fetal bovine serum, antibiotics, heparin (Upjohn) at 5 units/ml, and crude HBGF as described previously (16). Cultured human vascular smooth muscle cells (HVSMC) were a kind gift of Peter Libby (Brigham and Women's Hospital, Boston) and were grown in medium 199 containing 10% fetal bovine serum and antibiotics. Human monocytes, isolated from leukocyte-rich elutriates by adherence to plastic, were made quiescent by maintenance in RPMI 1640 medium containing 10% calf serum for 2 days. Human foreskin fibroblasts (HFF), monkey kidney Cos-7 cells, and human epidermoid carcinoma cells (A431; American Type Culture Collection) were grown in the same media as HVSMC.

Purification of RNA and Northern Blot Analysis. Total RNA purification and Northern blot analysis using the hybridization buffer system of Church and Gilbert have been described

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Abbreviations: Cox, cyclooxygenase; hCox, human Cox; PG, prostaglandin; IL-1, interleukin 1; PMA, phorbol 12-myristate 13-acetate; Dex, dexamethasone; LPS, lipopolysaccharide; HBGF, heparin-binding growth factor; HUVEC, human umbilical vein endothelial cells; HVSMC, human vascular smooth muscle cells; HFF, human foreskin fibroblasts; RT–PCR, reverse transcriptase–polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ORF, open reading frame; RA, rheumatoid arthritis.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M90100).

previously (17). The filters were hybridized under high stringency [65°C, 20% (vol/vol) formamide] with ^{32}P-labeled cDNA probes. The cDNA probes used were (*i*) hCox-1 cDNA probe, which extends from amino acid codon 1 to codon 378, and (*ii*) 2.5-kb hCox-2 cDNA probe, which extends from nucleotide 540 to nucleotide 3387 (see Fig. 1).

Cloning of hCox-2 cDNA. We used the degenerate oligonucleotides 5′-AARGARGTNYTNGARAARGT-3′ and 5′-CATYTCNACYTGNGTRTCYTT-3′ (R = A or G, Y = C or T, and N = A, G, C, or T) to PCR amplify the cDNA from NIH 3T3 cells. The resulting 300-base-pair (bp) fragment was subcloned, sequenced, and labeled with [^{32}P]dCTP to screen 10^6 plaque-forming units of λgt10 cDNA library prepared from PMA- and cycloheximide-treated HUVEC (17). The largest clone (2.5 kb) was characterized further by DNA sequencing. To obtain the full-length cDNA clone for hCox-2, we used the reverse transcriptase (RT)–PCR and primer extension–PCR procedures. First, we used the sequence conservation of Cox proteins to design a "guess" primer (5′-AATCCCTGTTGTTCCCTCCCATGCCAGAA-3′), which corresponds to the N-terminal sequence NPCCS-NPCQN, residues 19–28. The antisense primer (5′-TCATTTGAATCAGGAAGCTGCTTT-3′) corresponds to the hCox-2 sequence KKQLPDSNEI, residues 154–161, which was deduced from the 2.5-kb cDNA clone. RT–PCR analysis was done on HUVEC RNA for 35 cycles. The resulting 500-bp fragment was subcloned and sequenced. Second, we used the primer extension–PCR with sequences from the 500-bp hCox-2 cDNA sequence corresponding to the N terminus. Antisense primer (5′-CAGTTTTCTCCAT-AGAATCCTGTCCGG-3′) (RTGFYGEN, residues 45–53) was used to reverse transcribe PMA-treated HUVEC poly(A)$^+$ RNA, and the cDNAs were converted to blunt double-stranded forms by treatment with the Klenow fragment of *Escherichia coli* DNA polymerase and T4 DNA polymerase. The cDNAs were ligated to annealed adaptors (A, 5′-TAATACGACTCCGAATTCGG-3′ and B, 5′-CCGAATTCGG-3′) overnight. The ligated cDNAs were used as a template in a PCR that used the hCox-2-specific internal antisense primer (5′-AATCGCACTTATACTGGT-CAAATCCC-3′) (GFDQYKCD, residues 36–43) and adaptor A. A single band of 300 bp was produced, which was then subcloned and sequenced as well.

DNA Sequencing. The cDNAs were subcloned in pBS vector and unidirectional deletions were produced in both strands by the exonuclease III/mung bean nuclease method (18). The cDNAs were then sequenced by using the *Taq* DNA polymerase sequencing kit (Applied Biosystems).

RT–PCR. The RT–PCR protocol was used to quantify the hCox-1 and hCox-2 mRNAs (13). Specific primers for hCox-1 and hCox-2 are 5′-TGCCCAGCTCCTGGCCCGCCGCTT-3′ (sense) and 5′-GTGCATCAACACAGGCGCCTCTTC-3′ (antisense) and 5′-TTCAAATGAGATTGTGGGAAAAT-TGCT-3′ (sense) and 5′-AGATCATCTCTGCCTGAGTA-TCTT-3′ (antisense), respectively. Primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as internal controls, as described previously (12, 13). At 22 and 25 cycles of amplification, the amount of the amplified product is linear with respect to the input RNA for GAPDH and Cox primers, respectively (13). For quantitation of amplified DNA, Southern blot analysis was conducted with hCox-1 and -2 cDNA probes. Radioactivities in the Cox bands were normalized with respect to the GAPDH bands from parallel samples.

***In Vitro* Translation of hCox-1 and -2 mRNAs.** The 1800-bp open reading frames (ORFs) of the hCox-1 and -2 mRNAs were amplified from HUVEC mRNA by RT–PCR and cloned in the TA Cloning vector PCR1000 (Invitrogen, San Diego). The DNAs were linearized, and *in vitro* transcripts were produced by using the T7 RNA polymerase (Promega) and were translated in [^{35}S]methionine-supplemented rabbit reticulocyte lysate translation system (Promega).

For immunoprecipitation with antiserum to ovine Cox, 10 μl of the translation mixes were diluted in 90 μl of 0.05 M Tris·HCl-buffered saline, pH 7.8/0.1% Tween-20/10 mM EDTA, incubated with 1 μl of ovine Cox antiserum (19) for 1 h at 4°C, and immunoprecipitated with staphylococcal protein A-Sepharose beads. The immunoprecipitates were separated by electrophoresis on an SDS/10% polyacrylamide gel, fluorographed, and autoradiographed as above.

Expression of hCox-1 and -2 mRNAs in Cos-7 Cells. The ORFs of the hCox-1 and -2 cDNAs were subcloned in the eukaryotic expression vector pCDNA (Invitrogen) and transiently transfected into subconfluent Cos-7 cells by using the lipofectin reagent (BRL). The cells were scraped with a rubber policeman and resuspended in 0.5 ml of Dulbecco's modified Eagle's medium (DMEM) buffered with 0.05 M Hepes, pH 7.4. [1-^{14}C]Arachidonic acid (final concentration 25 μM) was then added to the cell suspension and incubated at 37°C for 15 min. The cells were pelleted, the medium was acidified and extracted with 6 vol of chloroform/methanol, 2:1 (vol/vol), and lipids were separated on a silica gel G TLC plates in the solvent system I_w and autoradiographed as described previously (3). In some experiments, the autoradiographs were analyzed by densitometry. The R_f values of the PGs were determined by comparison with authentic standards.

RESULTS AND DISCUSSION

Cloning of hCox-2 cDNA. The nucleotide sequence of hCox-2 is shown in Fig. 1. The 98-bp 5′ untranslated region is rich in G and C residues. The ORF begins with the sequence CTGCGATGC, which agrees with the rules proposed by Kozak (20) for the initiation of translation. The 604-amino acid long ORF is followed by a 3′ untranslated region of 1475 bases containing 12 copies of the ATTTA Shaw–Kamen sequence, a motif found in many immediate-early genes and shown to confer enhanced mRNA degradation (21).

The deduced amino acid sequence of hCox-2 is 61% identical to the hCox-1 protein. In contrast, it is more closely related to mCox-2 (88%) and chicken Cox-2 (81%) polypeptides. The salient features of the two Cox polypeptides are shown in Fig. 2. The putative N-terminal signal peptide region appears to be shorter than in hCox-1. However, two putative N-linked glycosylation sites at residues 53 and 130, the axial heme ligand His-295, putative transmembrane domain residues 277–292, the active-site residue Tyr-371, and the distal heme ligand His-374 are conserved between hCox-1 and hCox-2. In the C terminus of the polypeptide, the sequence surrounding Ser-516, the aspirin-acetylation site (22, 23) residue, is highly conserved. The hCox-2 polypeptide contains an 18-residue insertion containing a putative N-glycosylation site at the C-terminal region. The polypeptide ends with the sequence RSTEL, a sequence motif that resembles the KDEL endoplasmic reticulum-retention signal (24). Together, these results suggest that the hCox-2 cDNA encodes an enzymatically active Cox protein.

Expression of the hCox-2 cDNA. The ORFs of the hCox-1 and -2 cDNAs were subcloned in the eukaryotic expression vector pCDNA for expression in Cos cells. To determine if our cloned hCox-1 and -2 ORFs were translatable, we prepared capped *in vitro* transcripts by using T7 RNA polymerase. When these transcripts were translated in [^{35}S]methionine-supplemented reticulocyte lysates, equivalent levels of 70-kDa polypeptides were synthesized (Fig. 3*A*). Smaller polypeptides were synthesized as well, perhaps due to initiations inside the ORF and/or degradation of the full-length polypeptide. Furthermore, polyclonal antiserum against the ovine Cox specifically immunoprecipitated the two polypep-

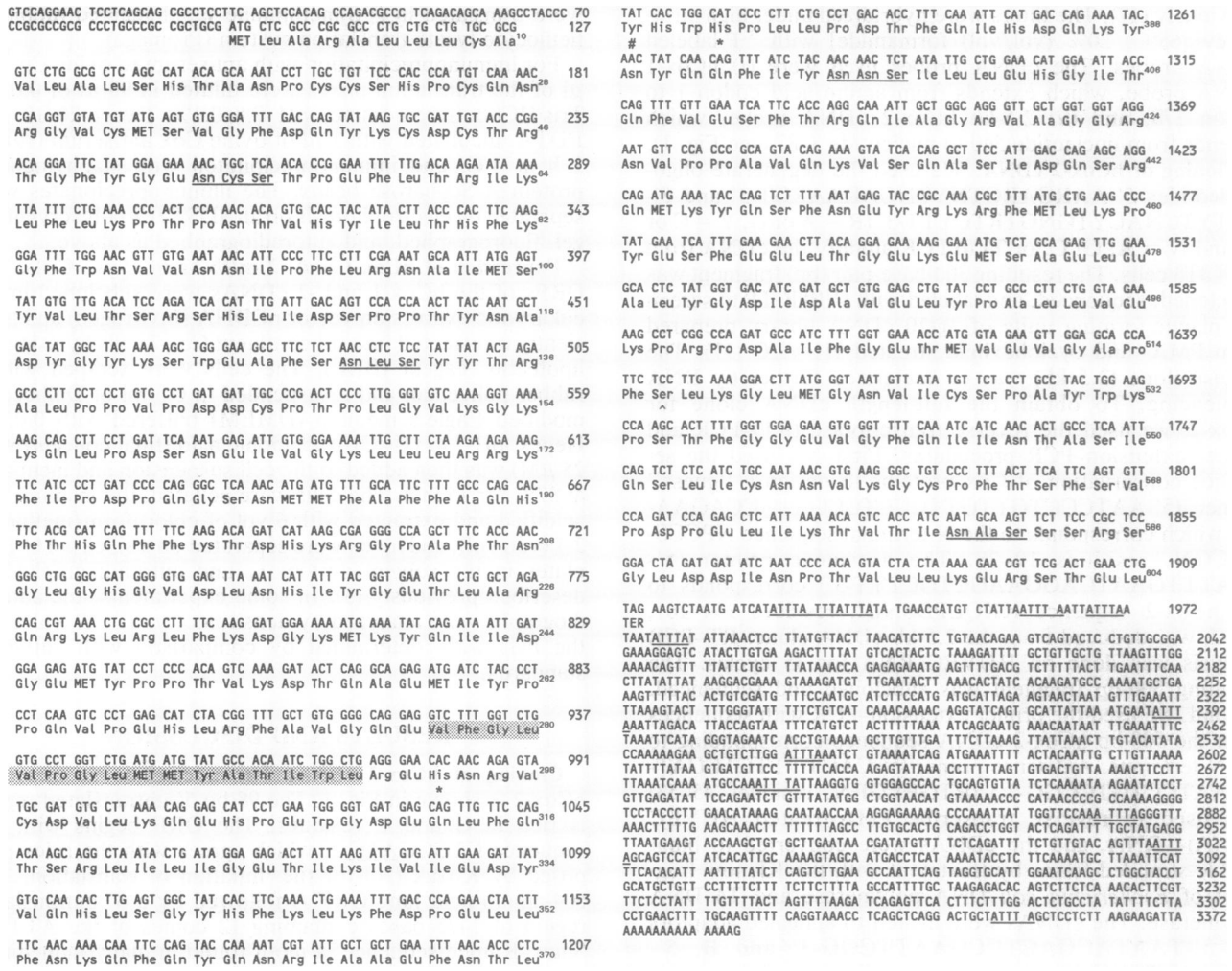

FIG. 1. Composite nucleotide sequence of hCox-2 cDNA and deduced amino acid sequence. Three overlapping clones of the hCox-2 cDNA were sequenced. Putative N-glycosylation sites are underlined, heme coordination sites are indicated with asterisks, the active site tyrosine is indicated with a #, the aspirin-acetylation site is indicated with an arrow, and the presumed transmembrane domain is shaded. In the 3′ untranslated sequence, ATTTA motifs are underlined.

tides (Fig. 3*B*). These experiments indicate that the hCox-1 and -2 ORFs are translationally competent and that the two polypeptides are immunoreactive with the polyclonal Cox antiserum. Transfection of the hCox-1 and -2 cDNAs into Cos cells, followed by the assay for the Cox enzyme—namely, incubation of cells with radioactive arachidonic acid and analysis by TLC—was conducted. Both Cox-1 and -2 transfected Cos cells expressed Cox enzyme activity; measured by the conversion of exogenous arachidonic acid into PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and hydroxy fatty acids (Fig. 3*C*). However, the Cox activity expressed by Cox-2-transfected cells was significantly lower. Interestingly, the hCox-2-transfected Cos cells synthesized lower relative levels of PGE_2 and hydroxy fatty acids. It is not known whether the quantitative difference in product profiles represents *bona fide* differences in enzymatic properties—i.e., differential coupling to isomerases—or whether the differences are due to nonenzymatic breakdown of high levels of PGH_2 in hCox-1-transfected cells. The hCox-2-transfected cells were pretreated with 10 μM indomethacin or 100 μM ibuprofen for 10 min at 37°C and challenged with [1-^{14}C]arachidonic acid, and the Cox activity was determined by laser densitometric quantitation of the autoradiographs. As shown in Table 1, indomethacin and ibuprofen inhibited the Cox activity in Cos cells transfected with both constructs. These data suggest that the enzymatic activity of the hCox-2 protein is sensitive to indomethacin and ibuprofen. Northern blot analysis of the transfected cells with Cox-1 and -2 probes indicated that the steady-state mRNA levels for Cox-2 were correspondingly lower (Fig. 3*D*), suggesting that the Cox-2 mRNA is highly unstable in Cos cells. It is surprising that motifs for RNA instability were present in the ORF; it is generally thought that the 3′ untranslated region contains information for RNA instability (21). Nevertheless, our data establish that the

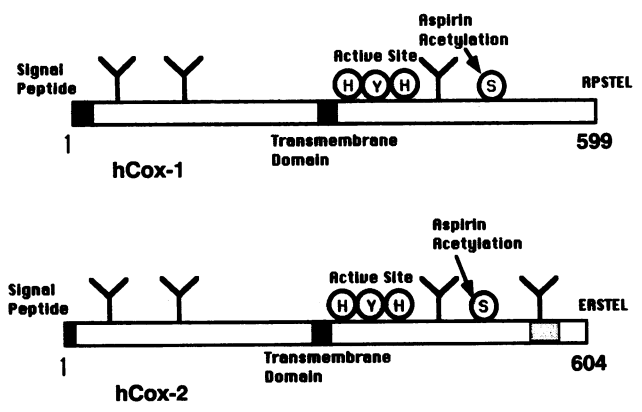

FIG. 2. Diagram of hCox-1 and -2 polypeptides. Salient features are indicated: Y, putative N-linked glycosylation sites; Ⓗ, histidine; Ⓨ, active-site tyrosine.

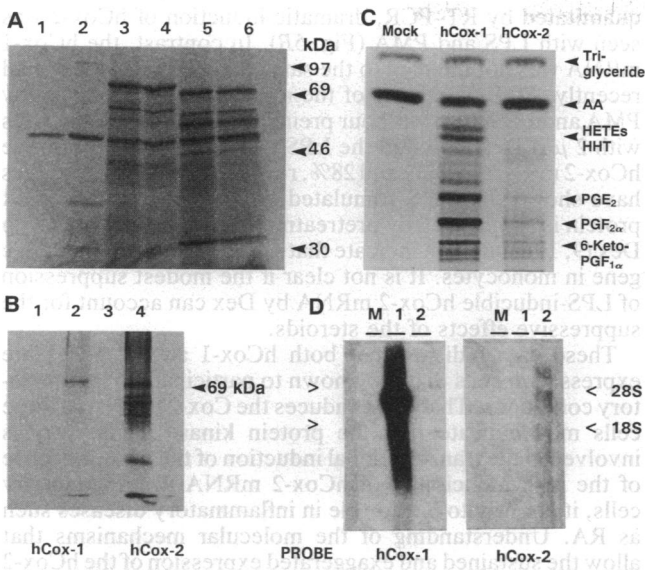

FIG. 3. Expression of hCox-1 and -2 polypeptides. (*A*) *In vitro* translation of hCox-1 and -2 mRNAs. Capped *in vitro* transcripts were translated in rabbit reticulocyte lysates supplemented with [^{35}S]methionine, and proteins were fractionated by electrophoresis on an SDS/10% polyacrylamide gel and autoradiographed. Lane 1, no RNA; lane 2, 1 μg of positive control amylase RNA; lane 3, 2 μg of hCox-1 mRNA; lane 4, 1 μg of hCox-1 mRNA; lane 5, 2 μg of hCox-2 mRNA; and lane 6, 1 μg of hCox-2 mRNA. (*B*) Immunoprecipitation of *in vitro* translated polypeptides. Equivalent radioactivities of *in vitro* translated hCox-1 and -2 polypeptides were immunoprecipitated with the ovine Cox antiserum (lanes 2 and 4) and nonimmune rabbit serum (lanes 1 and 3). Immunoprecipitates were fractionated on an SDS/10% polyacrylamide gel, fluorographed, and autoradiographed. (*C*) Enzymatic activity of transfected Cos cells. Subconfluent cultures of Cos-7 cells were transfected with the expression vector pCDNA containing cDNA inserts for hCox-1 and -2. Two days after transfection, the cells were harvested by scraping and incubated with [1-^{14}C]arachidonic acid (25 μM) for 15 min at 37°C. The medium was then extracted and analyzed by TLC and autoradiography. Mock-transfected cells were treated similarly but did not receive any exogenous DNA. AA, arachidonic acid; HETEs, hydroxyeicosatetraenoic acids; HHT, 12-hydroxyheptadecatrienoic acid. (*D*) RNA expression in transfected Cos cells. Cos-7 cell pellets that were transfected as above were pelleted, total RNA was purified, and 10 μg of RNA was analyzed by Northern blot analysis with hCox-1 and -2 cDNA probes. Lane M, mock transfected; lane 1, hCox-1; lane 2, hCox-2.

hCox-2 cDNA is capable of producing an enzymatically active Cox.

Regulation of hCox-1 and -2 mRNA Levels. It is well accepted that the products of the Cox pathway are involved in inflammatory conditions, especially in chronic inflammatory diseases such as rheumatoid arthritis (RA) (25). We have shown recently in animal models of RA as well as in arthritic patients that the extent and intensity of Cox expression directly correlates with disease severity (26). In these inflamed tissues, highest levels of immunoreactive Cox antigen are found in monocytes, endothelial cells, and synovial fibroblasts (26). Mononuclear infiltration, fibroblast proliferation, and enhanced angiogenesis by the resident endothelial cells are characteristics of chronic inflammatory diseases (25). To better define the *in vitro* regulation of the Cox genes, we next studied the expression of Cox-1 and -2 mRNAs in diploid human cells. The tumor promoter PMA inhibits the growth and induces differentiation of HUVEC *in vitro* and induces angiogenesis *in vivo* (27). In addition, PMA induces PG and Cox protein synthesis in HUVEC (28). We determined by Northern blot analysis the effect of PMA on the level of the transcripts for hCox-1 and -2. As shown in Fig. 4*A*, low levels of hCox-1 and -2 transcripts are expressed in quiescent HUVEC. Four hours of treatment with PMA and cycloheximide resulted in a strong induction of hCox-2 mRNA, whereas hCox-1 mRNA levels did not change appreciably. The kinetics of the PMA effect were studied by using RT–PCR. In Fig. 4*B*, the effect of PMA on the relative levels of hCox-1 and -2 mRNAs are quantified and plotted. While PMA induced a modest (2.8-fold) increase in hCox-1 mRNA, the effect on hCox-2 mRNA was highly significant (28-fold increase). The induction occurred early (1 h) and peaked at 6 h. These data indicate that (*i*) both hCox-1 and hCox-2 genes are expressed by HUVEC and (*ii*) PMA induces the Cox-2 gene to a much higher extent than the

Table 1. Inhibition of hCox-1 and -2 enzyme activity

Transfection of Cos-7 cells	[1-^{14}C]PG synthesis, arbitrary OD units		
	6-Keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$
Mock-transfected	<0.5	<0.5	<0.5
pCDNAhCox-1	66	121	116
+ 10 μM indomethacin	8	6	15
+ 100 μM ibuprofen	10	11	26
pCDNAhCox-2	6	3	9
+ 10 μM indomethacin	<0.5	<0.5	<0.5
+ 100 μM ibuprofen	<0.5	<0.5	4

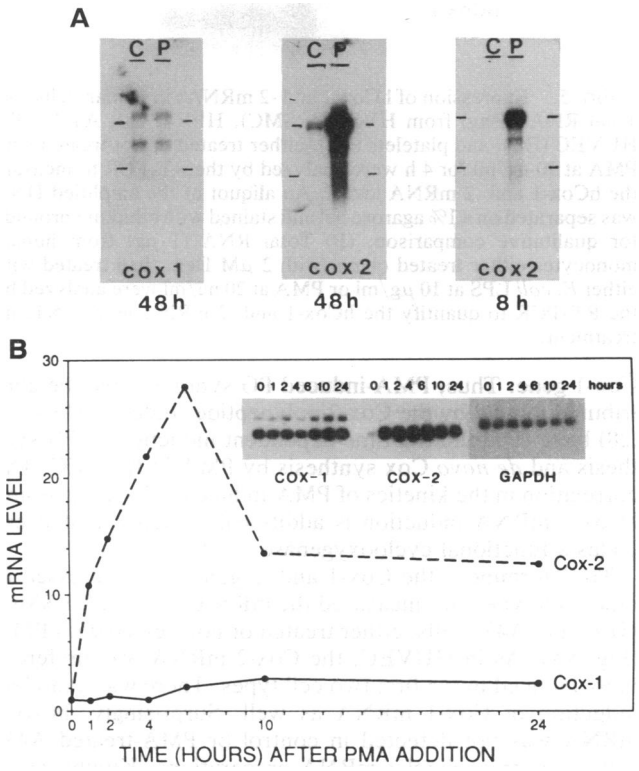

FIG. 4. Regulation of Cox-1 and -2 mRNAs by PMA in HUVEC. (*A*) Poly(A)$^+$ RNA (2 μg per lane) from quiescent HUVEC either treated (P) or not treated (C) with PMA at 20 ng/ml and cycloheximide at 10 μg/ml for 4 h was separated on a 1.2% agarose formaldehyde gel, and Northern blot analysis was conducted with hCox-1 and -2 cDNA probes. The exposure times of the blots to the x-ray film are indicated under the autoradiograms. The center and right autoradiograms are from the same blot exposed for different times. The positions of the 28S and 18S ribosomal RNA markers are indicated by lines. (*B*) Total RNA was purified from quiescent HUVEC treated with PMA at 20 ng/ml for the indicated amounts of time. It was then used (1 μg) in the RT–PCR assay to quantitate the levels of hCox-1 and -2 mRNAs. The levels of the mRNA are normalized with respect to GAPDH mRNA.

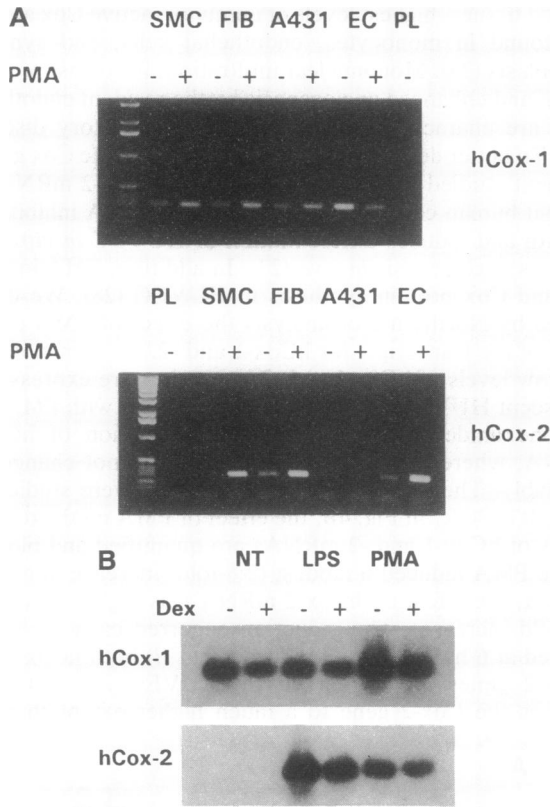

FIG. 5. Expression of hCox-1 and -2 mRNAs in human cells. (*A*) Total RNA (1 μg) from HVSMC (SMC), HFF (FIB), A431 cells, HUVEC (EC), and platelets (PL), either treated or not treated with PMA at 20 ng/ml for 4 h were analyzed by the RT–PCR to measure the hCox-1 and -2 mRNA levels. An aliquot of the amplified DNA was separated on a 1% agarose gel and stained with ethidium bromide for qualitative comparison. (*B*) Total RNA (1 μg) from human monocytes either treated or not with 2 μM Dex, then treated with either *E. coli* LPS at 10 μg/ml or PMA at 20 ng/ml were analyzed by the RT–PCR to quantify the hCox-1 and -2 mRNA levels. NT, no treatment.

Cox-1 gene. Thus, PMA-induced PG synthesis may be contributed mainly by the Cox-2 polypeptide. Indeed, Wu *et al.* (28) have reported the time-dependent induction of PG synthesis and *de novo* Cox synthesis by PMA in HUVEC. The correlation in the kinetics of PMA-induced PG synthesis and hCox-2 mRNA induction is additional evidence that it encodes a functional cyclooxygenase.

To determine if the Cox-1 and -2 genes are expressed in other cell types, we measured the mRNA levels in HVSMC, HFF, and A431 cells, either treated or not treated with PMA (Fig. 5*A*). As in HUVEC, the Cox-2 mRNA was preferentially induced in the first two cell types. There was a modest induction of Cox-1 mRNA as well. Surprisingly, hCox-2 mRNA was not detected in control or PMA-treated A431 cells or human platelet mRNA preparations. Kujubu *et al.* (15) have reported the restricted expression of the TIS10 (mCox-2) transcript in many immortalized cell lines derived from diverse tissues. All of the human cells tested that express the hCox-2 transcript as an inducible gene were normal diploid cells strains with a finite replicative life span. Further studies are required to determine the relationship between Cox-2 expression and cellular immortalization/transformation *in vitro*.

We also studied the expression of the Cox-1 and -2 mRNAs in normal peripheral blood monocytes. Quiescent monocytes that were pretreated with Dex for 2 h were stimulated with LPS or PMA for 5 h. When the levels of hCox-1 and -2 were quantitated by RT–PCR, dramatic induction of hCox-2 was seen with LPS and PMA (Fig. 5*B*). In contrast, the hCox-1 mRNA was not induced to the same magnitude. As reported recently, PGE_2 synthesis of the monocytes was induced by PMA and LPS (9). Two-hour preincubation of the monocytes with 2 μM Dex inhibited the LPS and PMA response on the hCox-2 mRNA by 32% and 28%, respectively. Recent studies have shown that LPS-stimulated *de novo* synthesis of Cox protein is suppressed by pretreatment of the monocytes with Dex (9, 10). Our data indicate that Cox-2 is the LPS-inducible gene in monocytes. It is not clear if the modest suppression of LPS-inducible hCox-2 mRNA by Dex can account for the suppressive effects of the steroids.

These data indicate that both hCox-1 and -2 genes are expressed in cells that are known to participate in inflammatory conditions. That PMA induces the Cox-2 mRNA in these cells may indicate that the protein kinase C pathway is involved in the transcriptional induction of the gene. Because of the high inducibility of hCox-2 mRNA in inflammatory cells, it is likely to play a role in inflammatory diseases such as RA. Understanding of the molecular mechanisms that allow the sustained and exaggerated expression of the hCox-2 gene may lend novel insights into the pathogenesis of inflammatory diseases.

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