Cyclooxygenase Is an Immediate-early Gene Induced by Interleukin-1 in Human Endothelial Cells*

(Received for publication, February 5, 1990)
Jeanette A. M. Maier‡, Timothy Hia§, and Thomas Maciag
From the Laboratory of Molecular Biology, Jerome H. Holland Laboratory for the Biomedical Sciences, American Red Cross, Rockville, Maryland 20855

The monokine interleukin-1 (IL-1) inhibits endothelial cell growth and induces prostacyclin production in human endothelial cells. Since cyclooxygenase (Cox) is the rate-limiting enzyme in the synthesis of prostanoids, we evaluated the ability of IL-1 to stimulate Cox expression by human umbilical vein endothelial cells (HUVEC) in vitro. Our data demonstrate that 1) the Cox mRNA is expressed at low levels in untreated cells; 2) IL-1α induces the Cox mRNA within 2 h, and this induction is sustained for more than 24 h; 3) IL-1α induction is dose-dependent; 4) cycloheximide potentiates the induction of the Cox mRNA by IL-1α while actinomycin D prevents the induction, and 5) IL-1α also stimulates Cox production in a time-dependent fashion which correlates with the increase in prostacyclin synthesis. These data suggest that Cox is an immediate-early gene induced by IL-1 in HUVEC and may contribute to the regulation of the endothelial cell differentiation pathway in vitro.

Endothelial cells comprise the lining of all blood vessels and contribute to organ physiology and to the pathology of human diseases. A few of the functions of the endothelial cell include the maintenance of vascular tone and the production of prothrombotic and anti-thrombotic activities among others (reviewed in Ref. 1). Inflammatory cytokines such as interleukin-1 (IL-1)1 are potent modulators of endothelial cell function. IL-1 inhibits the growth of endothelial cells (2) and alters the monolayer phenotype of the endothelial cell in vitro (3). Indeed, the endothelial cell assumes an elongated fibroblast-like phenotype in the presence of IL-1, a phenotype that is present during the very early stages of the endothelial

---

* This work was supported in part by National Institutes of Health Grants HL23248 and HL35627 and American Heart Association Grant-in-aid 881281 from the Maryland Affiliate (to T. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a fellowship from the Associazione Italiana per la Ricerca sul Cancro.
§ Supported by a postdoctoral fellowship from the National Arthritis Foundation.

1 The abbreviations used are: IL-1, interleukin-1; Cox, cyclooxygenase; RT, reverse transcription; PCR, polymerase chain reaction; HUVEC, human umbilical vein endothelial cells; PG12, prostacyclin; chx, cycloheximide; SDS, sodium dodecyl sulfate; kb, kilobase; PGE2, prostaglandin E2.

---

EXPERIMENTAL PROCEDURES

Cell Culture and Materials—Human umbilical vein endothelial cells (HUVEC) were a kind gift of M. Gimbrone (Harvard Medical School). Cells were serially passaged at 1:5 split ratios in Medium 199 (GIBCO) with 10% (v/v) fetal bovine serum (GIBCO), 50 μg/ml heparin, 150 μg/ml bovine crude heparin-binding (acidic fibroblast) growth factor-1 as described (36), and antibiotics in Costar (Cambridge, MA) tissue culture dishes coated with purified fibronectin (5 μg/cm²).
ments were performed with HUVEC passage 4–10. Recombinant human IL-1α was a kind gift of P. Lomedico, Hoffmann-La Roche (300 units/mg). The human Cox cDNA probe was isolated from a HUVEC λgt10 cDNA library using ovine cDNA as a probe. The cDNA clone extends from nucleotides 300 to 13.58 when compared HUVEC Xgt10 cDNA library using ovine cDNA as a probe. The (300 units/rig). The human Cox cDNA probe was isolated from a human IL-1α was a kind gift of P. Lomedico, Hoffmann-La Roche blotred with °P-random primer labeled human Cox cDNA (1 kb) at 65 °C for 20 h in 0.5% agarose gel containing 2.2 M formaldehyde (39), capillary blotted onto nylon membrane filters (Zeta Probe, Bio-Rad), and probed with °P-random primer labeled human cDNA Cox (1 kb) at 65 °C for 20 h in 0.5 M sodium phosphate containing 7% (w/v) SDS, 1% (w/v) bovine serum albumin, 1 mM EDTA, and 20% (v/v) formamide (40). Filters were washed at high stringency (0.1 × SSC, 65 °C) for 30 min and exposed to Kodak X-AR film for 24–72 h at −80 °C. All the filters were reprobed with human β-actin cDNA (41) to verify that equal amounts of RNA were loaded per lane.

Enzymatic Amplification Methods—Total RNA (1 μg) was incubated for 1 h at 37 °C with 200 units of Moloney murine leukemia virus reverse transcriptase using antisense primers (0.5 μM). The human cyclooxygenase or glyceraldehyde-3 phosphate dehydrogenase are the following: 5′-CCA GTC AGG TCC ACC-3′, 5′-TCT CCA ACG TGA G-3′ and 5′-GGC AAT GCG GTT GCG GTA TGC GGA CT-3′ (25). The sense and antisense primers used for glyceraldehyde-3 phosphate dehydrogenase are the following: 5′-CCA CCC ATG GCA ATT TCC ATG CCA-3′ and 5′-TCT AGT AGG GTC AGG TCC ACC-3′ (43).

Western blot Analysis—The cell culture medium of confluent HUVEC monolayers was replaced with Medium 199 and 5% (v/v) FBS for 20 h after which the cells were incubated for various times with 1 ng/ml IL-1α. At each time point the medium was removed, and the cells were washed with phosphate-buffered saline and scraped into 1 ml of phosphate-buffered saline. To solubilize Cox polypeptide, the pellets were sonicated in 50 mM Tris, pH 8.0, containing 10 mM EDTA, 10 mM MgCl2, 1 unit of RNasin, and 0.5 M NaCl, 3 mM MgCl2, 1 unit of RNasin, and 0.8 mM dNTPs. The reaction was heated to 95 °C for 5 min to separate the strands and diluted to 1 ml with TE (10 mM Tris–HCl, pH 7.5, 0.1 M NaCl). The reaction was then isolated by agarose electrophoresis and then transferred electrophoretically (400 mA, 2 h) to a nitrocellulose membrane in transfer buffer (25 mM Tris–HCl, pH 7.4, containing 200 mM glycine, 0.02% (w/v) SDS, and 20% (v/v) methanol) as described (44). After blocking the membrane for 2 h with 50 mM Tris–HCl, pH 7.4 (TBS) containing 150 mM NaCl and 5% bovine serum albumin, the membrane was incubated for an additional 2 h with 2% (w/v) goat anti-rabbit antibody purified from rabbit antiserum raised against ovine Cox (45). The filters were washed, incubated for 45 min with 4-10 rpm protein A/ml. The blot was washed four times with TBS containing 0.05% (v/v) Triton X-100 and the binding of 125I-protein A visualized by autoradiography with Kodak XAR films at −80 °C for 24 h.

Prostacyclin Synthesis—After starvation as previously described, HUVEC were stimulated with IL-1α (1 ng/ml) or cycloheximide (5 μg/ml) alone or in combination. Four hours later, the cells were harvested and RNA isolated. Poly(A)+ RNA (5 μg) was fractionated by formaldehyde-agarose gel electrophoresis, transferred to a nylon membrane and hybridized with °P-labeled human Cox cDNA as described under “Experimental Procedures.” Lane 1, untreated cells; lane 2, cycloheximide; lane 3, IL-1α; lane 4, IL-1α and cycloheximide. The filter was reprobed with a β-actin cDNA probe to demonstrate that similar amounts of RNA were loaded per lane. B, RNA was prepared from confluent HUVEC after 4 h of incubation with IL-1α (1 ng/ml) and/or actinomycin D (10 μg/ml). Total RNA (1 μg) was reverse-transcribed and amplified by PCR for 40 cycles as described under “Experimental Procedures.” The amplified products were separated on a 1% agarose gel and stained with ethidium bromide. Lane 1, untreated cells; lane 2, actinomycin D; lane 3, IL-1α; lane 4, IL-1α and actinomycin D; lanes 5–8, RNA was reverse-transcribed and amplified for glyceraldehyde-3-phosphate dehydrogenase to demonstrate that equal amounts of RNA were loaded.

RESULTS AND DISCUSSION

Since the monokine IL-1 induces prostacyclin synthesis in human endothelial cells (13, 14) and the enzymatic activity of Cox governs prostaglandin and prostacyclin production (17), we isolated a human Cox cDNA fragment (38) and used it to evaluate the expression of the Cox mRNA expression in HUVEC. Northern blot analysis of poly(A)+ RNA prepared from HUVEC incubated for 4 h with IL-1α (1 ng/ml) demonstrated that HUVEC express the Cox mRNA at low levels. Indeed, the Cox transcript was not detectable in 5 μg of poly(A)+ RNA derived from untreated cells (Fig. 1A, lane 1). However, following stimulation with IL-1α, the Cox mRNA was readily detectable as a single band having an approximate size of 3 kb (lane 3). The low abundance of Cox mRNA was also confirmed when total RNA was reverse transcribed and enzymatically amplified using Cox-specific oligonucleotide primers (Fig. 1B, lane 1). In contrast, HUVEC treated with IL-1α in the presence or in the absence of cycloheximide (chx; 5 μg/ml) demonstrated a superinduction of the Cox mRNA levels (Fig. 1A, lane 4), whereas chx alone did not appreciably alter the level of the Cox transcript (Fig. 1A, lane 2). Because the induction of the Cox mRNA by IL-1α could be due to an increase in the rate of transcription, stabilization of previously transcribed mRNAs, or a combination of both, the inhibition of transcription with actinomycin D, an inhibitor of RNA synthesis, was examined. The data shown in Fig. 1B demonstrate that actinomycin D (10 μg/ml) blocked IL-1α induction of the Cox mRNA (Fig. 1B). Since the level of Cox expression is relatively low, we used the reverse transcription-polymerase chain reaction (RT-PCR) method to assay for the presence of Cox mRNA. The products of the amplification were analyzed on a 1.0% agarose gel and stained with ethidium bromide. The oligonucleotide primers used for the specific amplification of Cox predict the generation of a 0.7-kb product, and a 0.7-kb band was readily detected (Fig. 1B). The 0.7-kb band strongly hybridized with radiolabeled Cox cDNA in a Southern blot (data not shown), confirming the identity of the 0.7-kb band as Cox. Further, amplification of the same

![Fig. 1](http://www.jbc.org/Downloadedfrom)
RNA with glyceraldehyde-3-phosphate dehydrogenase primers confirmed that equal amounts of RNA were reverse-transcribed. Serial dilution of RNA samples followed by RT-PCR assay demonstrated that Cox RNA was induced approximately 6-fold by IL-1a and chx after 4 h of incubation. Together, these data suggest that the increase in Cox mRNA expression by IL-1a may represent an early transcriptional event involved in endothelial cell activation. Nuclear run-on assays were used to determine whether the increase in Cox mRNA was regulated by a transcriptional or posttranscriptional mechanism. However, these attempts were without success due to the low abundance of the Cox mRNA in this system.

The effect of IL-1a on the expression of the Cox mRNA was dependent upon the concentration of IL-1a (Fig. 2, A and B). In addition, the potentiation of Cox mRNA expression by chx was observed at both concentrations of IL-1a (Fig. 2, A and B), an observation consistent with the suggestion that chx stabilizes the Cox mRNA. The kinetics of Cox mRNA expression in response to IL-1a (1 ng/ml) in the presence and in the absence of chx were also examined. As shown in Fig. 3, IL-1a induction of the Cox mRNA was time-dependent. At 2 h the induction was apparent, and it was sustained for 24 h. Furthermore, chx potentiated the ability of IL-1a to stimulate the expression of the Cox mRNA. It is interesting to point out that the effect of chx on the delayed transcriptional shut-off of the c-fos gene occurs rapidly (1-4 h), although the stabilization of the c-fos mRNA is sustained (47). We argue that a similar mechanism could explain chx potentiation of the Cox transcript by IL-1a in HUVEC cells.

To determine whether IL-1a also stimulates Cox production in HUVEC, we analyzed the level of the Cox polypeptide by Western blot analysis. As shown in Fig. 4, an increase in the level of the 70-kDa Cox polypeptide was observed in extracts prepared from HUVEC incubated with IL-1a (1 ng/ml). Accumulation of Cox was observed at 6 h, being maximal at 16 h. No band was observed in the Western blot format when the antibody was preincubated with purified ovine Cox (data not shown).

Table I shows the stimulation of PGI2 synthesis by HUVEC treated with IL-1a (1 ng/ml). PGI2 production was clearly induced after 8 h of incubation with IL-1a and was maximal after 24 h. The kinetics of IL-1a induction of Cox mRNA, Cox polypeptide, and PGI2 synthesis suggest that the IL-1a effect may be mediated mainly by its effect on de novo Cox synthesis in HUVECs. It is noteworthy that the effect of IL-1a on Cox mRNA appeared after 2 h and was sustained thereafter, while the increases in the level of Cox protein and enzyme activity appeared later (6-8 h). These results are in agreement with the studies in human dermal fibroblasts using inhibitors of transcription and translation (32), where the effect of IL-1a on Cox expression could temporally distinguish an early transcriptional phase as well as a late post-transcriptional phase. Because the increase in the Cox translational product appeared later, it is possible that IL-1a may induce additional post-transcriptional events in HUVEC. Recently, the regulation of Cox mRNA levels and prostaglandin synthesis by PDGF was described in NIH 3T3 cells. Interestingly, the increase of Cox mRNA occurred after maximal prostaglandin

| Incubation time with IL-1 | 6-Keto-PGF<sub><sub>1α</sub></sub> | pg/10<sup>5</sup> cells |
|--------------------------|----------------|-----------------|
| h                        |               |                 |
| 0                        | 35 ± 3        |
| 4                        | 57 ± 2        |
| 8                        | 88 ± 5        |
| 12                       | 110 ± 4       |
| 18                       | 200 ± 12      |
| 24                       | 290 ± 10      |

Fig. 2. The expression of the Cox RNA by HUVEC after 4 h of exposure to different concentrations of IL-1a. Northern blot analysis was performed as described under "Experimental Procedures." Lanes 1 and 2, control; lanes 3 and 4, 0.1 ng/ml IL-1a; lanes 5 and 6, 1 ng/ml IL-1a. Lanes 2, 4, and 6 received cycloheximide (5 μg/ml). B, polymerase chain reaction assay. The RT-PCR assay was performed in HUVEC treated with IL-1a (lanes 3-6 and 9-12) and 2 μg/ml cycloheximide (lanes 2, 4, 6, 8, 10 and 12). Lanes 1 and 2, control; lanes 3 and 4, 0.1 ng/ml IL-1a; lanes 7-12, RNA was reverse-transcribed and subsequently amplified for glyceraldehyde-3-phosphate dehydrogenase.

Fig. 3. The kinetics of Cox mRNA expression in response to IL-1a. Total RNA was isolated as a function of time from confluent HUVEC monolayers. RT-PCR was performed on these samples as described under "Experimental Procedures." A 123 4 5 6

Fig. 4. The kinetics of the human Cox by HUVEC treated with IL-1a. Confluent monolayers of HUVEC were incubated for different times with IL-1a (1 ng/ml). The cells were harvested, and Western blot analysis was performed using rabbit anti-ovine Cox as described under "Experimental Procedures."
IL-1 Induces Cox mRNA in HUVEC

synthesis was achieved, suggesting that platelet-derived growth factor stimulated prostaglandin synthesis is not dependent upon de novo Cox expression in 3T3 cells (35). The ability to detect the Cox transcript in total RNA samples extracted from untreated 3T3 cells demonstrates that Cox expression is relatively high in this cell line. In contrast, the low abundance of Cox mRNA in HUVEC could depend on the requirement for HBGF-1 in the cell culture system (36). Indeed HBGF-1 has been shown to reduce prostaglandin synthesis in HUVEC (47).

IL-1 has been shown to inhibit endothelial cell growth and alter the morphology of the endothelial-cell monolayer from the traditional cobblestone phenotype to a fibroblast-like cell shape in vitro (3). The later phenotype resembles the morphology assumed by the endothelial cell during the very early stages of the endothelial cell differentiation pathway in vivo (4, 5). A characteristic of the regulation of transcription during the immediate-early stages of the cell cycle for a variety of immediate-early genes includes the sensitivity to superinduction by chx (35, 48–51). Indeed, the rapid transcriptional activation of c-fos, c-jun, and c-myc in response to inductive signals and their superinduction by chx is well described (50) and occurs as a result of the inhibition of mRNA degradation as well as a prolongation of transcriptional shut-off. Thus, the superinduction of Cox by IL-1 and chx in HUVEC suggests that Cox is an immediate-early human endothelial cell differentiation-response gene. Since Cox is a key regulatory enzyme for the synthesis of prostanooids (18) and many of the biological effects of IL-1, such as pyresis, inflammation, and vasodilation, are prostanoid-mediated (52), these data may provide new insights into the molecular mechanisms utilized by IL-1 to regulate prostaglandin synthesis.

Acknowledgments—We thank Sally Young and Kitty Wawzinski for expert secretarial assistance and P. Lomedico for his generous gift of human IL-1α.

REFERENCES

1. Ryan, U. (1986) The Endothelial Cell, CRC Press, Inc., Boca Raton, FL.
2. Norioka, K., Harai, M., Kitani, A., Hirose, T., Hirose, W., Harigai, M., Suzuki, K., Kawakami, M., Tabata, H., Kawagoe, M., and Nakamura, H. (1987) Biochim. Biophys. Res. Commun. 145, 969–975.
3. Montesano, R., Orci, L., and Vassalli, P. (1985) J. Cell Physiol. 122, 424-434.
4. Macig, T., Kadish, J., Wiekins, L., Steemer, M. B., and Weinstein, R. (1982) J. Cell Biol. 94, 511–520.
5. Friesel, R., Komoriya, A., and Macig, T. (1987) J. Cell Biol. 104, 689-696.
6. Bevilacqua, M. P., Bober, J. S., Majeau, G. R., Cotran, R. S., and Gimbrone, M. A., Jr. (1984) J. Exp. Med. 160, 618-623.
7. Bevilacqua, M. P., Bober, J. S., Wheeler, M. F., Conran, R. E., and Gimbrone, M. A., Jr. (1985) Am. J. Pathol. 121, 394-403.
8. Nawroth, P. P., Handley, D. A., Emon, C. T., and Stern, D. M. (1986) Proc. Natl. Acad. Sci., U. S. A. 83, 3460-3464.
9. Dejana, E., Breviario, F., Erroi, A., Buscolino, F., Mussoni, L., Gramme, M., Pintucci, G., Casali, B., Dinarello, C. A., Van Damme, J., and Mantonvani, A. (1987) Blood 69, 683–699.
10. Emeis, J. J., and Kooistra, T. (1986) J. Exp. Med. 163, 1260-1266.
11. Nachman, R. L., Hajjar, K. A., Silverstein, R. L., and Dinarello, C. A. (1966) J. Exp. Med. 134, 1569-1600.
12. Bevilacqua, M. P., Schleiff, R. R., Gimbrone, M. A., Jr., and Laskowski, D. J. (1986) J. Clin. Invest. 78, 587-591.
13. Endo, H., Akahoshi, T. K., and Kashiwagi, S. (1988) Biochem. Biophys. Res. Commun. 154, 1007-1014.
14. Rossi, V., Breviario, F., Ghezzi, P., Dejana, E., and Mantonvani, A. (1986) Science 229, 174-176.
15. Moncada, S., Higgs, E. A., and Vane, J. R. (1977) Lancet 1, 18-20.
16. Wolfe, L. S. (1982) J. Neurochem. 38, 1-14.
17. Needleman, P., Turk, J., Jakschik, B. A., Morrison, A. R., and Laskowith, J. R. (1986) Proc. Natl. Acad. Sci., U. S. A. 83, 69-102.
18. Rollins, T. E., and Smith, W. L. (1980) Biochim. Biophys. Acta 60, 484-486.
19. Van Der Ouderaa, F. J., Boytenhak, M., Nugteren, D. H., and Van Dorp. P. A. (1977) Biochim. Biophys. Acta 487, 315-333.
20. Roth, G. J., Stanford, N., and Mayerus, P. W. (1975) Proc. Natl. Acad. Sci., U. S. A. 72, 3073-3076.
21. Wu, K. K., Hatzakis, H., Los, S. S., Seong, D. C., Sanduja, S. K., and Taylor, H. H. (1988) Proc. Natl. Acad. Sci., U. S. A. 85, 1943-1947.
22. Pagan, J., and Goldberg, A. L. (1986) Proc. Natl. Acad. Sci., U. S. A. 83, 2771-2775.
23. Smith, W. L., and Lands, W. E. M. (1972) Biochemistry 11, 3276-3280.
24. Henler, M. E., and Lands, W. E. M. (1980) J. Biol. Chem. 255, 10431-10436.
25. Yokoyama, C., and Tanabe, T. (1989) Biochem. Biophys. Res. Commun. 165, 888-894.
26. Bevilaqua, M. P., Majeau, G. R., Cotran, R. S., and Gimbrone, M. A., Jr. (1986) J. Lipid Res. 27, 54–61.
27. Yokota, K., Usaka, M., Oshima, T., Yamanoto, S., Kunihara, N., Yoshima, T., and Kumeegawa, M. (1986) J. Biol. Chem. 261, 1510–1514.
28. Habenicht, A. J. R., Goerig, M., Grulich, J., Rothe, D., Gronwald, R., Loter, U., Schettler, G., Kommerell, B., and Ross, R. (1985) J. Clin. Invest. 76, 1381-1387.
29. Cohn, L. M., Kortylewski, M., and MacDonald, P. C. (1988) J. Biol. Chem. 263, 7846-7854.
30. Frazier-Scott, K., Hatzakis, H., Seong, D., Jones, C. M., and Wu, K. K. (1988) J. Clin. Invest. 82, 1877-1883.
31. Ruiz, A., Wyche, A., Siegel, N., and Needleman, P. (1988) J. Biol. Chem. 263, 3022-3028.
32. Ruiz, A., Wyche, A., and Needleman, P. (1988) Proc. Natl. Acad. Sci., U. S. A. 85, 1097-1101.
33. Lin, A. H., Bienkowski, M. J., and Gorman, R. R. (1989) J. Biol. Chem. 164, 17379-17383.
34. Mercer, R., Bravo, R., Beach, S., and Casley, P. (1984) J. Cell Biol. 104, 1213-1221.
35. Smith, W. L., and Lands, W. E. M. (1972) Biochemistry 11, 121, 394-403.
36. Bevilacqua, M. P., Schleff, R. R., Gimbrone, M. A., Jr., and Laskowith, D. J. (1986) J. Clin. Invest. 78, 587–591.
37. Endo, H., Akahoshi, T. K., and Kashiwagi, S. (1988) Biochem. Biophys. Res. Commun. 154, 1007-1014.
38. Rossi, V., Breviario, F., Ghezzi, P., Dejana, E., and Mantonvani, A. (1986) Science 229, 174-176.
Cyclooxygenase is an immediate-early gene induced by interleukin-1 in human endothelial cells.
J A Maier, T Hla and T Maciag

J. Biol. Chem. 1990, 265:10805-10808.