Selective Expression of Mitogen-inducible Cyclooxygenase in Macrophages Stimulated with Lipopolysaccharide*

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Two forms of cyclooxygenase are known to be present in eukaryotic organisms: a cyclooxygenase (COX-1) first purified from ram seminal vesicles encoded by a 2.8-kilobase mRNA, and a newly discovered mitogen-inducible cyclooxygenase (COX-2) encoded by a 4-kilobase mRNA. Expression of these two forms of the enzyme in rat alveolar macrophages stimulated with lipopolysaccharide was investigated by 1) determining the activity of newly synthesized enzyme after inactivating the endogenous enzyme with aspirin; 2) comparing levels of newly synthesized enzyme proteins in cells treated with or without lipopolysaccharide; and 3) assessing the expression of the mRNAs encoding COX-1 and COX-2. Levels of enzyme proteins were assessed by Western blot analysis and immunoprecipitation of 38S-labeled enzyme using two different antibodies, one specific for COX-2 and the other recognizing both forms of the enzyme but preferentially recognizing COX-1. We report here that the enhanced cyclooxygenase activity induced by the bacterial lipopolysaccharide in rat alveolar macrophages is caused by selective expression of COX-2. Expression of COX-2 in macrophages stimulated by lipopolysaccharide was completely inhibited by dexamethasone, whereas COX-1 was unaffected. In resting unstimulated macrophages, only COX-1 but not COX-2 was detected. Levels of mRNA for the COX-2 in macrophages were increased, but those of the COX-1 were not affected by lipopolysaccharide as assessed by reverse transcription coupled with polymerase chain reaction. These results indicate that increased synthesis of prostaglandins and thromboxanes in lipopolysaccharide-stimulated macrophages results from selective expression of COX-2.

Prostaglandin (PG)\(^1\) H synthase/cyclooxygenase (EC 1.14.99.1) is a rate-limiting enzyme in prostaglandin and thromboxane synthesis. PGH synthase possesses two enzymatic activities: a cyclooxygenase which catalyzes the oxygenation of arachidonic acid to PGG\(_2\) and a peroxidase which reduces PGG\(_2\) to PGH\(_2\) (1). A unique feature of this enzyme is that following the conversion of PGG\(_2\) to PGH\(_2\), it is rapidly autoinactivated, leading to severe curtailment of product formation (2). Thus, stimulation of prostaglandin synthesis by agonists via the cyclooxygenase pathway is self-limiting because of this rapid inactivation of the enzyme. This fact raises an important question as to whether liberation of free arachidonic acid is sufficient to induce the burst of prostaglandin and thromboxane formation in many agonist-response systems. Growing evidence now indicates that rapid de novo synthesis of PGH synthase often is required to sustain the conversion of arachidonic acid to prostaglandins. It is well documented that enhanced synthesis of prostaglandins in cells stimulated by mitogens accompanies increased cellular expression of cyclooxygenase (3–5).

cDNA for cyclooxygenase was cloned, and the primary structure of the enzyme has been determined from the nucleotide sequence of the cDNA in sheep (6–8), mouse (9), and human (10). Recently, a mitogen-inducible form of cyclooxygenase encoded by a 4-kilobase mRNA (designated as COX-2, whereas the first discovered form of the enzyme encoded by a 2.8-kilobase mRNA is designated as COX-1 in this report) was discovered in chicken embryo fibroblasts transformed by Rous sarcoma virus (11) and in 3T3 cells stimulated by a phorbol ester or serum (12, 13). It has also been shown that dexamethasone selectively inhibits the expression of COX-2 in immortalized murine fibroblasts without affecting that of COX-1 (13, 14). The possibility of differential expression of these two forms of cyclooxygenase and the relative contribution of each form of enzyme to overall production of prostaglandins in agonist stimulated cells have not been investigated.

We studied the expression of these two forms of the enzyme in rat alveolar macrophages stimulated by endotoxin lipopolysaccharide (LPS), which is known to induce the expression of cyclooxygenase in human monocytes (5). The results show that unstimulated cells contained only COX-1 protein, whereas increased cyclooxygenase activity in LPS-stimulated cells was caused by selective expression of COX-2.

**MATERIALS AND METHODS**

Isolation of Macrophages—Rats (Sprague-Dawley) were kept in Duo-Ro Bioclean racks (Laboratory Products) with positive pressure to minimize exposure to airborne bacteria. Alveolar macrophages were collected by bronchoalveolar lavage as described by Chandler and Fulmer (15). Cell viability as determined by trypan blue exclusion was greater than 90%. More than 95% of lavaged cells were macrophages as determined by differential counting. Cells in RPMI 1640 (GIBCO) were allowed to adhere to 24-well microtiter plates (Costar) for 2 h (37 °C, 5% CO\(_2\)).

Incubation and Assay for PGH Synthase Activity—To study recovery of cyclooxygenase activity, cells were allowed to adhere in presence of aspirin (600 μM) in RPMI. Cells were incubated in RPMI contain-
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ing 3% fetal calf serum with or without LPS, (10 μg/ml, Difco) for 16 h. The medium was removed after the incubation, and cells were incubated in the fresh medium containing arachidonic acid (30 μM, Nu-Chek) for 10 min to determine cyclooxygenase activity as described by Fu et al. (5). Levels of PGE₂ were determined by radioimmunoassay.

Preparation of Cyclooxygenase Antibodies—Antibodies specific for COX-2 were prepared by immunizing rabbits with a 19-amino acid polypeptide conjugated to thyroglobulin (Sigma) according to the procedure described elsewhere (16). The amino acid sequence of the peptide was as follows: (Cys)-Ala-Ser-Ala-Ser-His-Ser-Leu-Asp-Ala-Pro-Thr-Val-Leu-Ile-Lys (carboxyl-terminal). The amino-terminal cysteine was added to conjugate the peptide to the carrier protein. This sequence is present only in the carboxyl-terminal region (amino acid sequence 581–598) of COX-2 (murine). The peptide was synthesized in the Biotechnology Core Laboratory of the Louis and Lillian Stulman Institute for Neurological Sciences (New Orleans). A second polyclonal antibody was prepared against purified rat seminal vesicle cyclooxygenase (Oxford Biomedical Research).

Metabolic Labeling and Immunoprecipitation of Cyclooxygenase— Cells were preincubated for 12 h in the medium containing LPS and then further incubated in 1 ml of methionine-free RPMI containing 100 μCi of [³⁵S]methionine (1,139 Ci/mmol, ICN) in Dulbecco's modified Eagle's medium (GIBCO) for 4 h. Cells were washed three times with phosphate-buffered saline, pH 7.4, and solubilized by sonicating in 200 μl of solubilization buffer (50 mM Tris, pH 7.4, 1% Tween 20, 0.5 mM MgCl₂, 2.5 mM KCl, 1 mg/ml bovine serum albumin) containing 3% fetal calf serum with or without LPS, (10 μg/ml, Difco) for 16 h. The medium was removed after the incubation, and cells were incubated with arachidonic acid (30 μM) for 30 min at 37 °C. The reaction was terminated by heating at 95 °C for 10 min, and COX activity was inactivated by sonication in 200 μl of solubilization buffer. Aliquots of PCR products were run on a 15% agarose gel in Tris borate/EDTA buffer as described elsewhere (19). Fractionated PCR products were capillary blotted onto nylon membrane filter (Zeta Probe, Bio-Rad) and probed with [³²P]-random primer-labeled murine COX-1 cDNA probe for COX-1 or with [³²P]-labeled oligonucleotide probe (5'-ATCTAGTCTGGAGTGCGGAGCATT-GCAT-3') for COX-2. This oligonucleotide probe was labeled with the [³²P]ATP 5'DNA terminus labeling system (Bethesda Research Laboratories) and is complimentary to a region encoding 18 amino acids found in COX-2 but not in COX-1. The sense and antisense primers used for gyceraldehyde-3-phosphate dehydrogenase mRNA as an internal standard were: 5'-GTGAACTGCTGGAAGCAGAGGATT-3' and 5'-CACAGCTTCTCTGAGTGCGGATGTGAT-3', respectively (20). This primer set yields the PCR product for 555 base pairs. The PCR amplification conditions for gyceraldehyde-3-phosphate dehydrogenase were 94 °C for 2 min for 1 cycle and 94 °C for 1 min and 63 °C for 5 min for 25 cycles. Fractionated and capillary blotted PCR product of gyceraldehyde-3-phosphate dehydrogenase mRNA was probed with [³²P]-labeled oligonucleotides (5'-GGCATCACCGGAGGGCGGAGATGATGC-3').

In Vitro Transcription and Translation—cRNA transcripts encoding COX-1 and COX-2 were synthesized using T7 RNA polymerase and COX-1 or COX-2 cDNAs cloned into Bluescript SK− vector as template. Translation of the cyclooxygenase mRNAs was done in rabbit reticulocyte lysate (Promega Co.) containing [³⁵S]methionine as described previously (21).

RESULTS AND DISCUSSION

Enhanced Recovery of Cyclooxygenase Enzyme Activity by LPS in Aspirin-treated Macrophages—After pretreatment of macrophages with aspirin (500 μM) for 2 h, the endogenous cyclooxygenase activity was inactivated by 80–97%, depending on the batch of cells. Since the endogenous enzyme was inactivated by aspirin prior to treating cells with LPS, the recovery of the enzyme activity reflects the activity of newly synthesized enzyme. The time course for the recovery of the enzyme activity in macrophages treated with LPS showed that the maximum activity was reached in 16 h (Fig. 1). The

![FIG. 1. Time course for the cyclooxygenase activity recovered in LPS-stimulated macrophages after pretreatment with aspirin.](image-url)
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recovery of the enzyme activity by LPS required the presence of serum in the medium. Thromboxane A₂ was the major cyclooxygenase-derived metabolite of arachidonic acid in rat alveolar macrophages as assessed by radioimmunoassay for thromboxane B₂. The capacity of unstimulated macrophages to synthesize thromboxane B₂ was four times greater than that of PGE₂ (79.5 ± 9.3 versus 20.8 ± 1.6 pg/μg protein, p < 0.05, n = 12). However, thromboxane A synthase undergoes a "suicide" inactivation during catalysis (22), and it is not known whether thromboxane A synthase can also be induced by LPS in macrophages. Thus, we selected the measurement of PGE₂ instead of thromboxane B₂ to assess cyclooxygenase activity.

Accumulated levels of PGE₂ in the incubation medium without added exogenous arachidonic acid (Fig. 2) reflected the amounts of PGE₂ synthesized from the endogenous substrate in macrophages during the entire incubation time. Accumulated levels of PGE₂ can be affected not only by the activity of cyclooxygenase but also by availability of the substrate and other factors modulating the cyclooxygenase activity and substrate availability. The rate of production of PGE₂ in the incubation medium in which exogenous arachidonic acid was incubated with macrophages in 10 min reflects the activity of the de novo synthesized enzyme at the end of the incubation with LPS. By adding excess exogenous arachidonic acid to macrophages, total cellular cyclooxygenase activity can be measured because the intracellular enzyme pool is saturated with the substrate and is functioning at Vₘₐₓ. Accumulated levels of PGE₂ paralleled the enzyme activity (Fig. 2), suggesting that increased levels of PGE₂ in macrophages treated with LPS are because of enhanced de novo synthesis of cyclooxygenase. This LPS-induced enzyme activity was completely suppressed by actinomycin D, indicating that induction of cyclooxygenase by LPS occurs at the transcriptional level. Similarly, the synthetic glucocorticoid dexamethasone completely inhibited recovery of cyclooxygenase activity (Fig. 2). Recently, it has been shown that dexamethasone selectively inhibits the expression of COX-2 without affecting that of COX-1 (13, 14). Complete inhibition of both the enhanced enzyme activity and accumulated PGE₂ by dexamethasone suggested that the recovery of enzyme activity induced by LPS is because of selective expression of COX-2. Since COX-2 protein or COX-2 mRNA was not detected in macrophages prior to LPS stimulation (see Figs. 4–7) the initial cyclooxygenase activity of macrophages prior to aspirin treatment reflects the activity of COX-1. The COX-2 activity induced by LPS was greater than the initial COX-1 activity (Fig. 2).

Selective de Novo Synthesis of COX-2 Induced by LPS—To determine whether LPS selectively induces COX-2 expression, antibodies specific for COX-2 (Ab₂) was prepared against an 18-amino acid peptide present only in COX-2. These antibodies did not cross-react with COX-1 (murine) as assessed by in vitro transcription and translation and immunoprecipitation (Fig. 3). The second antibody (Ab₁+2) prepared using purified ram seminal vesicle cyclooxygenase recognized both COX-1 and COX-2 but preferentially recognized COX-1 as shown in Fig. 3.

Ab₂ did not detect ram seminal vesicle cyclooxygenase in Western blot analysis (Fig. 4), indicating that the vast majority of ram seminal vesicle cyclooxygenase is COX-1 or that the amino acid sequence of the peptide against which our Ab₂ was made is different from that found in sheep COX-2. Comparison of total cellular COX-1 and COX-2 by Western blot analysis of macrophages treated with LPS and/or dexamethasone also showed that COX-2 was present only in cells treated with LPS and/or dexamethasone or actinomycin D. Cyclooxygenase activity was determined as the amount of COX-1 and COX-2 immunoprecipitated with COX-1 and COX-2 antibodies (Ab₁+2) and as the amount of COX-1 immunoprecipitated with Ab₁+2 preimmune serum; lane 7, COX-2 immunoprecipitated with Ab₁+2 preimmune serum; lane 5, COX-2 immunoprecipitated with Ab₁+2 preimmune serum; lane 6, COX-1 immunoprecipitated with Ab₁+2 preimmune serum. Markers indicate position of standard proteins in kDa.

**FIG. 2.** Effects of cycloheximide (CHX, 2 μM), dexamethasone (DEX, 2 μM), and actinomycin D (ACT, 2 μg/ml) on cyclooxygenase activity and accumulated levels of PGE₂ in LPS-stimulated macrophages after pretreatment with aspirin. Aspirin-pretreated cells as described in the legend for Fig. 1 were incubated for 16 h with or without LPS, cycloheximide, dexamethasone, or actinomycin D. Cyclooxygenase activity was determined as described in the legend for Fig. 1. Accumulated levels of PGE₂ synthesized from endogenous arachidonic acid were determined from the incubation medium removed prior to adding exogenous arachidonic acid to macrophages. Initial activity represents endogenous cyclooxygenase activity in cells prior to aspirin treatment. Results are the mean ± S.E. (n = 6–12). Values with the same letter are not significantly different (Duncan’s multiple range test, p < 0.05).

**FIG. 3.** In vitro transcription and translation. cRNA transcripts encoding COX-1 and COX-2 were prepared using T7 RNA polymerase and COX-1 or COX-2 cDNAs cloned into Bluescript-vector template. Translation of the cyclooxygenase mRNAs was carried out in rabbit reticulocyte lysate (Promega) containing [³⁵S]methionine. In vitro translation products were visualized by SDS-polycrylamide gel electrophoresis followed by fluorography. Lane 1, COX-2 (translation product); lane 2, COX-1 (translation product); lane 3, COX-2 immunoprecipitated with COX-2 antibodies (Ab₂); lane 4, COX-1 immunoprecipitated with Ab₂; lane 5, COX-2 immunoprecipitated with Ab₁+2 preimmune serum; lane 6, COX-1 immunoprecipitated with Ab₁+2 preimmune serum; lane 7, COX-2 immunoprecipitated with cyclooxygenase antibodies prepared against ram seminal vesicle cyclooxygenase (Ab₁+2); lane 8, COX-1 immunoprecipitated with Ab₁+2 preimmune serum; lane 9, COX-2 immunoprecipitated with Ab₁+2 preimmune serum; lane 10, COX-1 immunoprecipitated with Ab₁+2 preimmune serum. Markers indicate position of standard proteins in kDa.

**FIG. 4.** Western blot analysis of cyclooxygenase in macrophages treated with or without LPS and/or dexamethasone (DEX). Rat alveolar macrophages were incubated in RPMI with 3% serum for 16 h with or without LPS and/or dexamethasone. Western blot analysis was carried out as described under "Materials and Methods." The blot was cut at the place between lanes 6 and 7. The blot containing lanes 1–6 was treated with the antibodies prepared against purified ram seminal vesicle cyclooxygenase (Ab₁+2), whereas the blot containing lanes 7–12 was treated with COX-2 antibodies (Ab₂). Lanes 1 and 7, cells prior to incubation; lanes 2 and 8, cells incubated in RPMI with 3% serum and LPS; lanes 3 and 9, cells incubated in RPMI with 3% serum, LPS, and dexamethasone; lanes 4 and 10, cells incubated in RPMI with 3% serum; lanes 5 and 11, cells incubated in RPMI with 3% serum and dexamethasone; lanes 6 and 12, ram seminal vesicle cyclooxygenase.
treated with LPS (Fig. 4). Induction of COX-2 was inhibited if dexamethasone was included in the incubation medium. In contrast, cyclooxygenase protein was detected from all samples when Ab1+2 was used. These results indicate that endogenous cyclooxygenase present in cells prior to aspirin and/or because of selective expression of COX-2.

Immunoprecipitation assays were used to evaluate directly de novo synthesis of COX-1 and COX-2 following LPS treatment. In these assays, immunoprecipitation by Ab2 showed that COX-2 was detected only in LPS-treated macrophages. Furthermore, incubating the LPS-treated cells with dexamethasone resulted in the complete disappearance of COX-2, indicating that dexamethasone inhibited de novo synthesis of this isoenzyme (Fig. 5A). Immunoprecipitation data obtained using Ab1+2, which can detect both COX-1 and COX-2, showed a pattern similar to that observed with Ab2 except that induction of cyclooxygenase by LPS appeared to be less pronounced (Fig. 5B). This is because Ab1+2 reacts primarily against COX-1 (Fig. 3) and contains only a low titer or low affinity antibody against COX-2. The diminished induction of cyclooxygenase when assessed by Ab1+2 further indicates that LPS selectively induced only COX-2 expression. Taken together, the activity data (Fig. 2), the Western Blot data (Fig. 4), and immunoprecipitation data (Fig. 5) show that LPS-induced cyclooxygenase activity results from COX-2 induction with little, if any, contribution from COX-1.

While performing the immunoprecipitation experiments, it was noticed that cyclooxygenase bands detected by Ab2 were always doublets. The intensity of these doublet bands was markedly attenuated by the addition of the COX-2-specific 18-amino acid peptide to the sample prior to the immunoprecipitation (data not shown). Whether these double bands imply the presence of an additional form of cyclooxygenase, COX-2 containing uncleaved signal peptide, alternatively spliced COX-2, or COX-2 that has been post-translationally modified is not known. In contrast to COX-2, the cyclooxygenase bands detected by Ab1+2 in Western blot analysis for macrophages without LPS treatment were always singlet except in LPS-treated cells in which Ab1+2 cross-reacts with induced COX-2 giving multiple bands.

**Increased Abundance of mRNA for COX-2 but Not COX-1 following LPS Treatment of Macrophages**—The nucleotide sequence of the antisense primer used to amplify COX-2 mRNA by RT-PCR was selected from a region encoding 18 amino acids which is found in the carboxyl-terminal region of COX-2 but not in COX-1. Thus, the primer pair would selectively amplify COX-2 mRNA. Additionally, to demonstrate that RT-PCR-synthesized fragments were indeed amplified from COX-2 mRNA and were not spurious PCR-generated products, an oligonucleotide probe was hybridized to the RT-PCR product. This oligonucleotide hybridized to a COX-2 nucleotide sequence located between the priming sites of the PCR, which shared no overlap with either primer. The probe for COX-2 showed no cross-reactivity with COX-1 under the hybridization conditions used. The time course for the RT-PCR product for COX-2 mRNA (Fig. 6) showed that the level of mRNA started to increase in response to LPS within 30 min of incubation and reached the maximum in 4–6 h. However, the time course for immunoprecipitated COX-

**Fig. 5.** Immunoprecipitation of cyclooxygenase from macrophages treated with or without LPS or dexamethasone (DEX). Rat alveolar macrophages were preincubated for 12 h with or without LPS (10 μg/ml), dexamethasone (2 μM), or both and then further incubated in methionine-free RPMI containing 100 μCi of [35S]methionine for 4 h. Cells were solubilized in the sodium dodecyl sulfate buffer as described under "Materials and Methods." Aliquots of the samples with equal amounts of radioactivity were preclibrated with the preimmune serum, immunoprecipitated with cyclooxygenase antibodies, and subjected to SDS-polyacrylamide gel electrophoresis and fluorography. The gel for panel A was exposed to the film for 12 h, whereas that for panel B was exposed for 3 days. The amount of [3H]-labeled ram seminal vesicle cyclooxygenase loaded to the gel for panel B was one-third of that loaded to the gel for panel A. In panel A COX-2 antibodies (Ab2) were used, whereas in panel B antibodies (Ab1+2) prepared with ram seminal vesicle cyclooxygenase, which recognizes both COX-1 and COX-2, were used. Lane 1, cells incubated in RPMI with 3% serum and LPS; lane 2, cells incubated in RPMI with 3% serum, LPS, and dexamethasone; lane 3, cells incubated in RPMI in 3% serum; lane 4, cells incubated in RPMI with 3% serum and dexamethasone; lane 5, [3H]acetyl-cyclooxygenase standard.

**Fig. 6.** Time course for levels of mRNA for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), COX-2, and COX-1 as assessed by RT-PCR in macrophages treated with and without LPS, and immunoprecipitated COX-2 protein in macrophages treated with LPS. Rat alveolar macrophages were incubated with or without LPS for specified times. Total RNA was extracted and amplified by RT-PCR as described under "Materials and Methods." Fractionated PCR products on a 1% agarose gel were capillary blotted onto nylon membrane filter and probed with [32P]-labeled COX-1 cDNA and [32P]-labeled oligonucleotides for COX-1 and COX-2 mRNA, respectively. The abundance of glyceraldehyde-3-phosphate dehydrogenase mRNA as an internal standard was also assessed by RT-PCR. The sequences of the primer sets and oligonucleotide probes for COX-1, COX-2, and glyceraldehyde-3-phosphate dehydrogenase mRNA were described under "Materials and Methods." Immunoprecipitation of COX-2 was described in the legend for Fig. 5 except that macrophages were preincubated with LPS for specified times and then metabolically labeled with [35S]methionine for 2 h instead of 4 h. The last lane in the COX-2 protein time course shows displacement of immunoprecipitated [35S]-labeled COX-2 (16 h sample) by the COX-2 peptide against which COX-2 antibodies were prepared. COX-2 protein was not detected in macrophages incubated without LPS at all time points. Thus, only immunoprecipitated COX-2 in macrophages incubated with LPS is shown.
2 protein showed that the maximum synthesis occurs around 14 h (12-h preincubation plus 2-h metabolic labeling) of incubation; thus, this time course parallels to that of the cyclooxygenase activity shown in Fig. 1. In spite of the presence of conspicuous amounts of COX-2 mRNA in samples incubated without LPS, COX-2 protein was not detected in these samples. The time gap between time courses for COX-2 mRNA and immunoprecipitated COX-2 protein and the absence of COX-2 protein in cells incubated without LPS imply the possibility that enhanced expression of COX-2 by LPS is mediated through post-translational events (i.e. expression of cytokines). Levels of mRNA for GADPH and COX-1 were not affected by LPS throughout the incubation period.

LPS treatment increased the abundance of COX-2 mRNA greater than 20-fold as assessed by RT-PCR and Southern blot analysis, and this increase was abolished by dexamethasone (Fig. 7). Resting macrophages prior to incubating with LPS or dexamethasone did not show detectable levels of COX-2 mRNA. COX-1 mRNA was detected in all samples, and its level was not affected by LPS or dexamethasone (Fig. 7). These results are consistent with those of Western blot analysis (Fig. 4) and immunoprecipitation data (Fig. 5). Detectable but low levels of COX-2 mRNA in macrophages incubated in the medium (RPMI plus 3% fetal calf serum) without LPS are probably a result of marginal stimulation rendered by the serum in the medium.

Results presented above indicate that enhanced cyclooxygenase activity in rat alveolar macrophages treated with LPS is caused by selective expression of COX-2 rather than COX-1. Whether similar results could be observed in other cell types and whether other agonists also selectively induce the expression of COX-2 in these cells remain to be determined.

During endotoxic shock there is an increased synthesis of prostaglandins (23, 24). Glucocorticoids are known to inhibit the release of eicosanoids in vivo in LPS-induced endotoxemia and to improve survival from endotoxic shock (25). Preferential inhibition of LPS-induced cyclooxygenase activity as compared with basal unstimulated cyclooxygenase activity by dexamethasone was also demonstrated by Fu et al. (5). Based on these results they hypothesized the presence of an LPS-inducible enzyme whose synthesis is sensitive to glucocorticoid inhibition. Furthermore, Needleman and his colleagues (26) also reported that peritoneal macrophages obtained from adrenalectomized mice showed a 2-3-fold induction in cyclooxygenase synthesis when compared with sham controls, suggesting that endogenous glucocorticoids can regulate the induction of cyclooxygenase. Our results indicate that LPS induces selective expression of COX-2 and that dexamethasone selectively inhibits the expression of COX-2, which provides the basis at least in part for the efficacy of glucocorticoids in improving survival from endotoxic shock.

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Addendum—While this manuscript was under review, O'Sullivan et al. (27) reported that rabbit alveolar macrophages primed with LPS and subsequently stimulated with opsonized zymosan showed enhanced induction of COX-2 mRNA (with no mRNA for COX-1 detected) and cyclooxygenase protein as assessed by using antibodies raised against sheep cyclooxygenase consisting of primarily COX-1.

REFERENCES


Fig. 7. RT-PCR analysis of mRNA for COX-1 and COX-2 in macrophages treated with or without LPS or dexamethasone (DEX). Rat alveolar macrophages were incubated with or without LPS, dexamethasone, or both for 4 h. RT-PCR was carried out as described in the legend for Fig. 6. Panel A, ethidium bromide-stained 1% agarose gel for PCR products of reverse-transcribed cDNAs showing expected fragments of 555, 450, and 583 base pairs (bp) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), COX-1, and COX-2 respectively. Panel B, Southern blot analysis of corresponding PCR products shown in panel A. Lane 1, cells prior to incubation; lane 2, cells incubated in RPMI with 3% serum and LPS for 4 h; lane 3, cells incubated in RPMI with 3% serum, LPS, and dexamethasone for 4 h; lane 4, cells incubated in RPMI with 3% serum for 4 h; lane 5, cells incubated in RPMI with 3% serum and dexamethasone for 4 h.

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