Cyclooxygenase-1 and -2 Expression in Rheumatoid Synovial Tissues

Effects of Interleukin-1β, Phorbol Ester, and Corticosteroids

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Abstract

High levels of immunoreactive cyclooxygenase (Cox; prostaglandin H synthase) are present in synovia from patients with rheumatoid arthritis (RA). We now show that the recently identified inducible isoform of Cox, Cox-2, is expressed in synovia from patients with RA. To further explore modulation of the Cox isoforms in RA synovial tissues, we examined the expression and modulation of Cox-1 and -2 in rheumatoid synovial explant cultures and cultured rheumatoid synovial fibroblast-like cells (synoviocytes). Immunoprecipitation of in vitro labeled proteins and Western blot analysis demonstrated the presence of both Cox-1 and -2 under basal conditions in freshly explanted rheumatoid synovial tissues. De novo synthesis of Cox-2 polypeptide was enhanced by IL-1β or PMA, and dramatically suppressed by dexamethasone (dex). Cox-1 expression, under the same conditions, showed only minor variation. Since mRNA for Cox-2 is highly unstable, we examined the regulation of Cox-2 transcripts in cultured rheumatoid synoviocytes. Under basal conditions both Cox-1 and -2 mRNAs were present at low levels, but Cox-2 mRNA was markedly increased by treatment with IL-1β or PMA. dex markedly suppressed the induction of Cox-2 mRNA. In sharp contrast, Cox-1 transcripts were not modulated by IL-1β or dex. These data suggest that modulation of Cox-2 expression by IL-1β and corticosteroids may be an important component of the inflammatory process in synovial tissues from patients with RA. (*J. Clin. Invest.* 1994. 93:1095–1101.) Key words: rheumatoid arthritis • inflammation • glucocorticoid • interleukin-1 • prostaglandin

Introduction

Prostaglandins (PGs) are important mediators of inflammation whose synthesis is initiated by release of arachidonic acid from cell membranes. Arachidonate is then converted to PGH_2 by cyclooxygenase (Cox),[1] or PGH synthase, the central enzyme in the PG synthetic pathway (1). The recent identification of a second isoform of Cox, Cox-2, has prompted new questions about the regulation of PG synthesis (2–7). Data from in vitro studies suggest that the expression of the Cox-2 gene is regulated by many extracellular stimuli, whereas Cox-1 is constitutively expressed (4–11).

PGs are important mediators of inflammation in RA (12, 13). The nonsteroidal antiinflammatory drugs (NSAIDs), which are inhibitors of Cox, are used extensively in the treatment of RA. The main products of the Cox pathway in rheumatoid synovia, namely PGE_2, $PGF_{2\alpha}$, thromboxane A_2, and prostacyclin, have multiple effects (14). For example, PGE_2 is produced by rheumatoid synovial tissues and probably plays a key role in the erosion of cartilage and juxtaarticular bone (15, 16).

IL-1 is a major proinflammatory cytokine present in synovial fluid and peripheral blood of patients with RA. Systemic levels of IL-1β have been found to correlate with disease activity (17). The majority of IL-1 is produced by macrophages present in inflamed rheumatoid synovia; however, synovial fibroblasts can also synthesize IL-1 (18). Injection of IL-1 into joints can induce inflammatory synovitis (19). One mechanism through which IL-1 exerts its proinflammatory effects is by increasing PG production in joint tissues (20, 21).

The inflammation of RA is extremely sensitive to corticosteroids. In fact, the dramatic clinical improvement of RA after corticosteroid treatment led early investigators to hypothesize that RA developed as a consequence of adrenal insufficiency (22). One effect of corticosteroids in RA is to reduce the production of PGs by synovial tissues, but the mechanism by which PG production is reduced in these tissues is not completely clear. It was thought that the effects of corticosteroids were mediated through the inhibition of phospholipase A_2 (PLA_2), the enzyme responsible for the release of arachidonate from the cell membrane. However, recent in vitro data suggest that the inhibition of both Cox and cytosolic PLA_2 expression may be important in the corticosteroid inhibition of PG production (23, 24).

Before the identification of Cox-2, our groups demonstrated with immunohistochemical techniques that Cox is increased in synovial tissues from patients with RA as compared with synovial tissues from patients with osteoarthritis or normal subjects (25). Additionally, Lewis rats with streptococcal cell wall (SCW)- or adjuvant-induced arthritis exhibit markedly increased immunostaining for Cox compared with untreated Lewis rats or arthritis-resistant rat strains. Treatment with dexamethasone (dex) results in decreased inflammation and Cox immunostaining in rats with SCW- or adjuvant-induced arthritis (25). To extend these data, we evaluated the expression of Cox-2 in RA synovial tissues using Cox-2-specific antisera. We also examined the expression of Cox-1 and -2 in freshly explanted rheumatoid synovial tissues and cultured

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1. *Abbreviations used in this paper:* Cox, cyclooxygenase; dex, dexamethasone; NSAID, nonsteroidal antiinflammatory drugs; SCW, streptococcal cell wall.

rheumatoid synoviocytes, and their modulation by IL-1β, PMA, and dex.

Methods

Transfection and in vitro metabolic labeling of Cox-1 and -2 cDNAs in Cos cells. The cDNAs for Cox-1 and -2 in the eukaryotic expression vector pCDNA (Invitrogen, San Diego, CA) were transfected into subconfluent monkey kidney Cos-7 cells as previously described (6). 48 h after transfection, the medium was replaced with methionine/cysteine-free DME containing L-^{35}S-methionine (> 1,000 Ci/mmol) and L-^{35}S-cysteine 1 mCi/well (> 600 Ci/mmol) (New England Nuclear, DuPont, Wilmington, DE), dialyzed fetal bovine serum (10%), and 10 mM Hepes (pH 7.0), and labeled for 2 h. Some cultures were coincubated with 5 μg/ml of tunicamycin.

Collection of tissues. Synovia were collected from patients with RA at the time of total joint replacement and placed immediately on ice for culture or in 10% formalin for immunohistochemistry. All patients met the American College of Rheumatology (1987) revised criteria for RA (26). No attempt was made to classify patients on the basis of disease duration, disease activity, or therapeutic regimen.

Immunohistochemistry. Formalin-fixed tissues were embedded in paraffin and sectioned (6 μm) onto gelatin-coated slides. Immunoperoxidase staining was done with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) as previously described in detail (25). The Cox-2-specific antisera were prepared by coupling the unique COOH-terminal peptide of human Cox-2 protein to keyhole limpet hemocyanin and injection into rabbits according to standard techniques (27). Anti-Cox-2 antisera or normal rabbit serum were used at dilutions of 1:100. Specificity was determined by preadsorption of the anti-Cox-2 antiserum with the Cox-2 COOH-terminal peptide (1 mg/ml) before staining.

Rheumatoid synovial explant culture. Synovial tissues specimens from three different RA patients for synovial explant culture were minced aseptically and placed in six-well culture plates ($\sim$ 0.5 gm/well) in 3 ml DME supplemented with 20% dialyzed fetal bovine serum (Gibco Laboratories, Grand Island, NY), Hepes (1 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) with or without dex (Sigma Chemical Co., St. Louis, MO), and incubated for 24 h at 37°C, 5% CO_2. Explant tissues from one patient were then treated with 10 ng/ml IL-1β (Genzyme, Cambridge, MA) or 20 ng/ml PMA (Sigma Chemical Co.) with or without dex 10^{-6} M for 4 h, divided, and placed either in Tri-Reagent™ (Molecular Research Center, Inc., Cincinnati, OH) for RNA extraction or frozen on dry ice and stored at −80°C for protein extraction. Synovial explant tissues from two other patients were metabolically labeled as above and treated, as indicated, with 10 ng/ml IL-1β (Genzyme) or 20 ng/ml PMA (Sigma Chemical Co.), with or without dex from 10^{-9} to 10^{-6} M, and incubated for 4 h. Tissues were then harvested and immediately frozen on dry ice.

Synoviocyte culture. Portions of each synovial specimen were digested for 4 h with 4 mg/ml collagenase (Type III; Worthington Biochemical, Freehold, NJ) in DME at 37°C in 5% CO_2. After digestion, the dissociated cells were centrifuged at 500 *g*, resuspended in DME supplemented with 10% fetal bovine serum (Gibco Laboratories), 10% human AB serum (BioWhitaker, Inc., Walkersville, MD), penicillin (100 U/ml), and streptomycin (100 μg/ml), and plated in 75-cm^2 flasks. The synovial fibroblast-like cells (synoviocytes) were allowed to reach 95% confluency ($\sim$ 10 d), then harvested with trypsin/EDTA (Gibco Laboratories), and passaged 1:3 into 175-cm^2 flasks. Thereafter, the cells were passaged 1:3 when they reached 95% confluency. Cells were used between the third and sixth passages.

If dex treatment was used, synoviocytes were pretreated for 24 h. The medium was changed, and cells were treated for 6 h with substances as indicated in the figure legends: IL-1β (1 or 10 ng/ml), PMA (20 ng/ml), dex (10^{-9}–10^{-6} M), indomethacin (2 μM), actinomycin D (10 μg/ml), and cycloheximide (5 μg/ml) (the last three from Sigma Chemical Co.).

Metabolic labeling of synoviocytes with L-^{35}S-methionine and -cysteine for immunoprecipitation analysis was performed as described above. Concomitant treatments were as indicated in the figure legends.

Immunoprecipitation. Metabolically labeled Cos-7 cells or rheumatoid synoviocytes were lysed by gentle extraction for 30 min at 4°C in RIPA buffer (50 mM Tris/HCl, pH 7.8, 10 mM EDTA, 1% Tween-20, 0.5% NP-40, 0.5% deoxycholic acid, 0.01% SDS, and 150 mM NaCl), and labeled synovial explant tissues were homogenized in RIPA buffer and cleared by centrifugation. The cell lysates and tissue extracts were trichloroacetic acid precipitated, and equivalent counts of the radiolabeled polypeptides were used for immunoprecipitation. The extracts were precleared with 10 μl of nonimmune rabbit serum and protein A–Sepharose beads for 1 h. Precleared lysates were then immunoprecipitated with a 1:100 dilution of polyclonal anti-Cox antibody (25) or specific anti-Cox-2 antisera. The complexes were separated on an SDS-PAGE (10% acrylamide) and visualized by autoradiography.

Western blot analysis. Synovial explants were homogenized in RIPA buffer as above. Proteins were separated on a 10% SDS-PAGE, transferred onto a nitrocellulose membrane, probed with the Cox-2-specific antisera, and visualized by the enhanced chemiluminescence detection system (Amersham Corp., Arlington Heights, IL).

RNA preparation and analysis. Total RNA was prepared from explant tissues or synoviocytes by the Tri-Reagent™ method (Molecular Research Center, Inc., Cincinnati, OH). For northern analysis, 20 μg of total RNA was subjected to electrophoresis through a 1.5% agarose/4% formaldehyde gel and transferred onto a nylon membrane (Nytran; Schleicher & Schuell, Inc., Keene, NH). PCR was used to generate cDNA fragments specific for human Cox-1 and -2 from reverse-transcribed human umbilical vein endothelial cell total RNA using the PCR primers as previously described (6). A human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA fragment was purchased from Clontech (Palo Alto, CA). These fragments were subsequently labeled by the random prime method (Boehringer-Mannheim Corp., Indianapolis, IN). Membranes were hybridized in 50% formamide at 42°C for 24 h with 2–5 × 10^6 cpm/ml cDNA probe. Washing was performed twice for 15 min in 2× SSC, 0.1% SDS, and twice for 15 min in 0.1× SSC, 0.1% SDS at 55°C for Cox-2 and G3PDH or 65°C for Cox-1. The membranes were exposed to Kodak XAR film for up to 2 wk at −80°C with intensifying screens. Densitometry was performed using a scanner from Molecular Dynamics (Sunnyvale, CA). Cox-1 and -2 were normalized to G3PDH detected on the same Northern blot for quantitation.

Results

Immunoprecipitation of Cox-1 and -2 polypeptides. The human Cox-1 and -2 polypeptides are 61% identical in primary sequence; however, the Cox-2 polypeptide contains an additional N-linked glycosylation site within the unique COOH-terminal insert region (6). To resolve the two Cox isotypes, Cos-7 cells were transfected with Cox-1 or -2 cDNAs, metabolically labeled, lysed, and immunoprecipitated with Cox antisera. We used both polyclonal Cox antisera prepared against ovine seminal vesicle Cox and cross-reacting with both Cox polypeptides (6) and specific anti-Cox-2 antisera prepared against a Cox-2 peptide. As shown in Fig. 1, the polyclonal antisera precipitated both Cox-1 and -2 polypeptides. The Cox-1 antigen consisted of a major band at 69 kD, whereas the Cox-2 polypeptide was composed of three bands at 69, 71, and 73 kD. The smallest Cox-2 band (69 kD), which was a minor band in Cox-2-transfected cells, was of the same molecular mass as the Cox-1 band. The major band in the Cox-2-transfected cells was 71 kD, and the top two bands (71 and 73 kD) were unique to the Cox-2 polypeptides. The anti-Cox-2 antisera precipitated only the Cox-2 polypeptide, confirming the

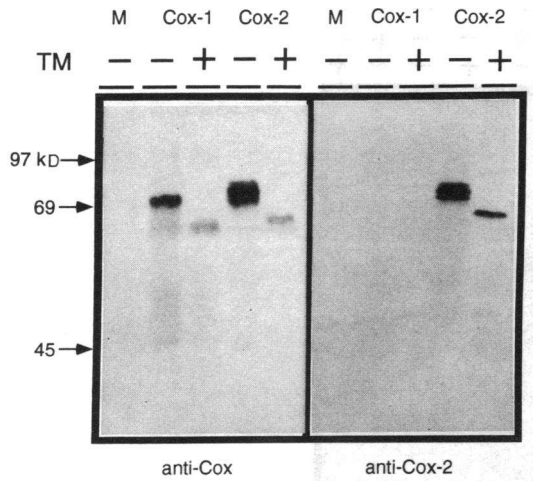

Figure 1. Immunoprecipitation of Cox-1 or -2 polypeptide from transfected Cos cells. Subconfluent cultures of Cos-7 cells were transfected with an expression vector containing either Cox-1 or -2 cDNA and metabolically labeled with L-^{35}S-methionine and -cysteine. Cell lysates were immunoprecipitated with polyclonal Cox antiserum as described in Methods. No complexes were detected in mock-transfected (*M*) Cos-7 cells.

specificity of the antibody (Fig. 1). The heterogeneity in the Cox-2 polypeptides is likely to be due to differential degrees of glycosylation. Indeed, in the presence of tunicamycin, a single band of ~ 69 kD was seen in both Cox-1- and -2-transfected cells (Fig. 1).

Immunohistochemistry. Cox-2 was readily detected in synovial tissues of patients with RA (Fig. 2). Immunostaining with anti-Cox-2 antisera revealed Cox-2 in infiltrating mononuclear cells, endothelial cells of blood vessels, and subsynovial fibroblast–like cells. In contrast, the synovial lining cell layer stained weakly. The immunostaining appeared to be localized to the perinuclear region and the endoplasmic reticulum. An identical pattern of staining was observed with anti–mouse Cox-2 antisera (provided by Dr. D. DeWitt, Michigan State University, East Lansing, MI) (28).

Adjacent sections stained with normal rabbit serum were negative. Extent and intensity of immunostaining of sections stained with anti-Cox-2 antisera previously incubated with the synthetic peptide from the COOH-terminal insert region of Cox-2 (1 mg/ml) were negative or markedly decreased (data not shown).

De novo synthesis of Cox-1 and -2 polypeptides. To study the regulation of Cox-1 and -2 polypeptides in rheumatoid synovium, freshly excised RA explants were labeled with L-^{35}S-methionine and -cysteine, and extracts were immunoprecipitated with the Cox antiserum as described in Methods. Untreated rheumatoid synovial tissue explants synthesized both Cox-1 and -2 polypeptides; however, treatment of the explants ex vivo with IL-1β or PMA for 4 h resulted in marked enhancement of Cox-2 synthesis. When the explants were pretreated with the antiinflammatory steroid, dex, the stimulatory effect of IL-1β and PMA on Cox-2 polypeptide expression was abolished (Fig. 3 *A*). Under these treatment conditions, the 69-kD band, which consists mainly of the Cox-1 polypeptide, was not stimulated above control levels by PMA or IL-1β. These data suggest that the de novo synthesis of Cox-2 in the RA synovial tissues is upregulated by inflammatory mediators and is suppressed by corticosteroids. To examine the dose response of dex suppression on de novo synthesis of Cox-2 polypeptides, the RA explants were pretreated with different concentrations of dex from 10^{-9} to 10^{-6} M for 24 h, stimulated with IL-1β or PMA, labeled, and immunoprecipitated. As shown in Fig. 3 *B*, dex at physiologically relevant concentrations completely inhibited Cox-2 synthesis. Using Cox-2-specific antisera, we confirmed by Western analysis that IL-1β and PMA increased expression of Cox-2 polypeptide and dex abolished Cox-2 expression (Fig. 4).

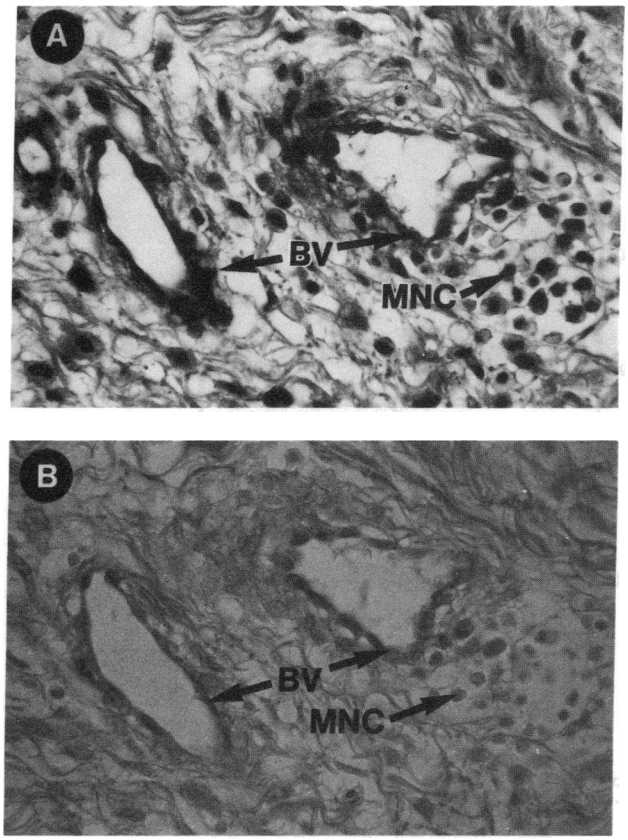

Figure 2. Immunostaining of rheumatoid synovia with anti-Cox-2 antisera. Representative synovial tissue sections of synovia from a patients with RA were stained with anti-Cox-2 antisera as described in Methods. Positive staining was indicated by brownish-black deposits. The background was light green on the original sections. In these black and white photographs, positive staining is indicated by black deposits. (*A*) RA synovium stained with anti-Cox-2 antiserum (×630 on original photograph). (*B*) Adjacent section stained with nonimmune rabbit serum (×630). *BV*, blood vessels; *MNC*, mononuclear inflammatory cells.

Immunoprecipitation of Cox-1 and -2 from metabolically labeled rheumatoid synoviocytes using both anti-Cox polyclonal antisera and specific anti-Cox-2 antisera also showed a marked increase in Cox-2 expression after treatment with IL-1β or PMA (Fig. 5). Dex suppressed IL-1β-induced expression of Cox-2. Both antisera gave a similar pattern on immunoprecipitation, suggesting that the majority of newly synthesized Cox is, in fact, Cox-2. So, in contrast to synovial explant tissues, cultured rheumatoid synoviocytes synthesize very little Cox-1 (compare Figs. 3 and 5). Treatment with tunicamycin revealed a single 69-kD polypeptide in synoviocytes similar to transfected Cos-7 cells (Figs. 1 and 5).

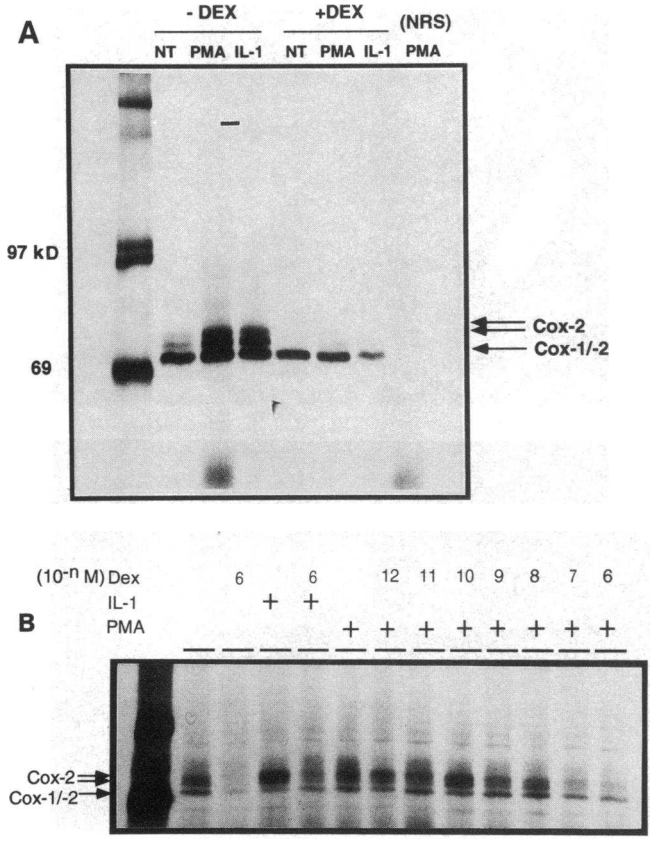

Figure 3. Immunoprecipitation of Cox-1 and -2 polypeptide from RA synovial explant cultures. (*A*) RA synovial tissue explants were treated with IL-1β (10 ng/ml) or PMA (20 ng/ml) with or without dex (10^{-6} M) pretreatment as indicated, and labeled with L-^{35}S-methionine and -cysteine. Tissue extracts were immunoprecipitated with polyclonal Cox antiserum or nonimmune rabbit serum. Bands specific for Cox-1 and -2 are identified by arrows. *NT,* not treated; *NRS,* nonimmune rabbit serum. (*B*) Cox immunoprecipitation assay was conducted as above on synovial explants, from a different patient with RA, pretreated with different concentrations of dex as indicated, and treated with IL-1β or PMA as above. Bands specific for Cox-1 and -2 are identified by arrows. *C,* control unstimulated synovial explants.

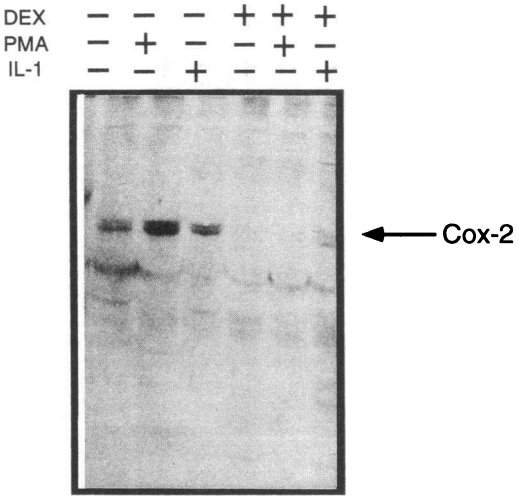

Figure 4. Western blot analysis of Cox-1 and -2 polypeptides in RA synovial explant cultures. RA explant cultures were pretreated with 10^{-6} M dex for 16 h and treated with either medium alone, IL-1β, or PMA for 4 h. The explants were then homogenized, and Cox-2 polypeptide detected by anti-Cox-2 antiserum as described in Methods.

RNA analysis of cultured rheumatoid synoviocytes. We examined in vivo rheumatoid synovial tissues for Cox-2 mRNA by quantitative reverse transcriptase PCR analysis. Cox-1 mRNA was detected in samples from five different RA patients; however, only low levels of Cox-2 mRNA were detected (data not shown). We suspect that the low levels of Cox-2 transcripts may be due to the rapid degradation in vivo of the Cox-2 mRNA, which has a high turnover rate ($\tau_{1/2}$ = 60 min) in vitro (Ristimäki, A., S. Garfinkel, J. Wessendorf, T. Maciag, and T. Hla, manuscript submitted for publication). In addition, it is also likely that endogenous corticosteroids may have contributed to the downregulation of the Cox-2 mRNA in vivo. We therefore evaluated the regulation of the Cox-1 and -2 transcripts by Northern analysis in cultured rheumatoid synoviocytes. Cox-1 and -2 transcripts were detectable under basal growth conditions, which included 20% serum. IL-1β increased Cox-2 mRNA levels in rheumatoid synoviocytes by > 10-fold in all experiments (Figs. 6 and 7). IL-1β-induced Cox-2 mRNA expression was suppressed by dex, but not by indomethacin (Fig. 6). PMA also markedly increased Cox-2 transcripts, and dex concentrations of 10^{-9}–10^{-6} suppressed PMA-induced Cox-2 mRNA (Fig. 6, and data not shown). Cox-1 mRNA was not stimulated by IL-1β, but increased modestly after treatment with PMA. Dex did not suppress basal or stimulated Cox-1 mRNA levels and Cox-1 transcript was unaffected by treatment with indomethacin (Fig. 6).

Basal and IL-1β-stimulated Cox-2 mRNA expression was completely suppressed by actinomycin-D. Cycloheximide alone increased the level of Cox-2 transcript, but did not affect IL-1β-induced Cox-2 mRNA expression (Fig. 7). In sharp contrast, neither actinomycin-D nor cycloheximide affected Cox-1 transcript levels (Fig. 7).

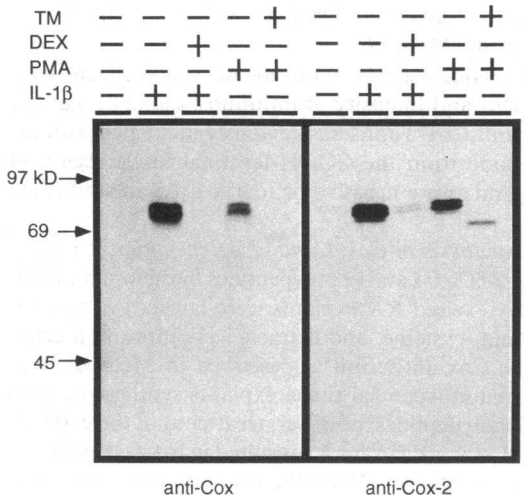

Figure 5. Immunoprecipitation of Cox-1 and -2 from cultured rheumatoid synoviocytes. Quiescent rheumatoid synoviocytes were pretreated with dex for 16 h or not as indicated, then stimulated with IL-1 or PMA for 4 h. Some cultures also received tunicamycin (5 μg/ml). The cells were metabolically labeled with ^{35}S-methionine and -cysteine, then the cell extracts were immunoprecipitated with either anti-Cox antiserum or anti-Cox-2 antiserum.

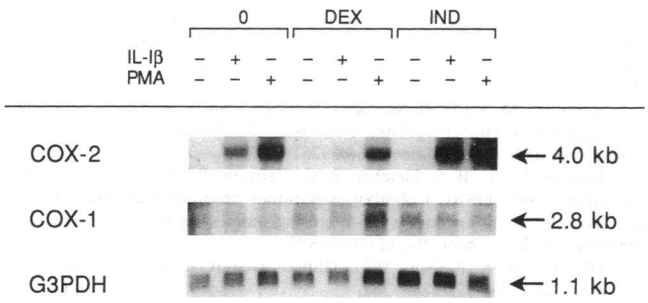

Figure 6. Northern analysis of Cox-1 and -2 mRNA in cultured rheumatoid synoviocytes. Total RNA (20 μg) was isolated from rheumatoid synovial fibroblasts cultured for 6 h in the presence of IL-1β (10 ng/ml) or PMA (20 ng/ml) as indicated, in the presence or absence of pretreatment with dex (10^{-6} M) for 24 h or indomethacin (2 μM). The filters were hybridized with each probe sequentially. The sizes of the transcripts are indicated. *IND,* indomethacin.

Discussion

Our groups previously demonstrated the presence of high levels of immunoreactive Cox expression in vivo in patients with RA (25). In light of the recent description of a second isoform of Cox that is induced by cytokines/mitogens and suppressed by corticosteroids in vitro, this study was designed to evaluate the expression of Cox-2 in rheumatoid synovia and to examine modulation of the Cox isoforms by IL-1β, PMA, and corticosteroids in freshly explanted rheumatoid synovial tissues and cultured rheumatoid synoviocytes. Cox-2 polypeptide is present in vivo in synovial tissues from patients with RA as demonstrated by immunohistochemistry with Cox-2-specific antibody. At present, we cannot quantitatively determine the relative amounts of Cox-1 polypeptide as compared with Cox-2 in these tissues by immunohistochemistry. Low levels of Cox-2 mRNA, as compared with Cox-1 mRNA, were detectable in these tissues by reverse transcriptase PCR analysis. The discrepancy in expression of Cox-2 mRNA and protein is most probably due to the very rapid degradation of Cox-2 mRNA (Ristimäki, A., S. Garfinkel, J. Wessendorf, T. Maciag, and T. Hla, manuscript submitted for publication). Cox-1 mRNA is a more stable transcript and was readily detectable in rheumatoid tissues (data not shown). Due to the differences in mRNA turnover between Cox-1 and -2, steady state mRNA levels in these in vivo tissues are unlikely to reflect the relative levels of the enzymatically active Cox isoforms.

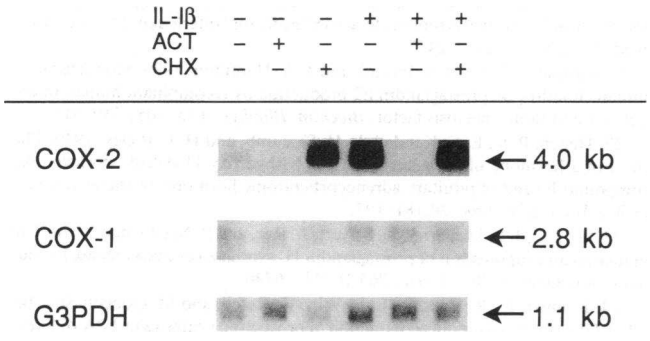

Figure 7. Effects of actinomycin-D and cycloheximide on Cox-1 and -2 mRNA expression. Northern analysis of 20 μg total RNA isolated from rheumatoid synovial fibroblasts cultured for 6 h in the presence of IL-1β (1 ng/ml), actinomycin D (10 μg/ml), and/or cycloheximide (5 μg/ml) as indicated. The filters were hybridized with each probe sequentially. The sizes of the transcripts are indicated. *ACT,* actinomycin-D; *CHX,* cycloheximide.

To examine the modulation of the Cox isoforms, we used freshly isolated rheumatoid synovial explant cultures and cultured rheumatoid synoviocytes. Cox-1 and -2 polypeptides were synthesized under basal conditions in explant tissues. However, Cox-2, but not Cox-1, synthesis was markedly increased by IL-1β or PMA and consistently suppressed by dex. Since rheumatoid synovia consist, in part, of massively hyperplastic synovial lining cells and subsynovial fibroblasts, we also studied cultured synoviocytes isolated from rheumatoid tissues. These cells expressed both Cox-1 and -2 mRNA and polypeptides under basal culture conditions, and IL-1β or PMA stimulated marked increases of Cox-2, but not Cox-1. Additionally, Cox-2, but not Cox-1, mRNA and polypeptide levels were suppressed by dex. Cox-2, but not Cox-1, mRNA exhibited a high turnover rate, since actinomycin-D eliminated both stimulated and unstimulated Cox-2 mRNA expression. Actinomycin-D had no effect on Cox-1 transcript levels under basal or stimulated culture conditions. Cycloheximide alone increased Cox-2 mRNA, a finding that was also reported by O'Banion et al. (7) in both mouse fibroblasts and human peripheral blood monocytes.

With the discovery of a new isoform of Cox, previous reports of Cox regulation are difficult to interpret. In most studies, conclusions were drawn based on measurements of PG synthesis, Cox activity, and protein expression as determined using polyclonal antibodies; however, mRNA levels were only infrequently reported (reviewed in reference 1). PMA increases Cox-1 mRNA in 3T3 fibroblasts (29), a response that we also noted in some of our experiments in rheumatoid synoviocytes. In human umbilical vein endothelial cells, IL-1α was shown to increase Cox-1 mRNA and protein expression (30). We, however, did not see an increase of Cox-1 protein in synovial tissue explant cultures or Cox-1 mRNA in cultured rheumatoid synoviocytes after treatment with IL-1β. Our data clearly implicate a marked induction of Cox-2 expression as a mechanism by which IL-1β stimulates PG production in rheumatoid synovial tissues and cells.

Masferrer et al. (31, 32) showed that LPS, which stimulates production of cytokines such as IL-1 and TNF-α, increases the Cox polypeptide synthesis in peritoneal macrophages. They further demonstrated that this stimulated Cox, but not basal Cox, expression could be suppressed by both endogenous and exogenous glucocorticoids. The authors inferred that the modulated Cox activity and protein expression in macrophages is due to Cox-2 (32). Their inference is supported by our data and by reports of Cox-2 induction by cytokines and/or mitogens and Cox-2 suppression by corticosteroids in several other cell types (4–6, 8–11).

The data reported here show that corticosteroids dramatically suppress Cox-2 expression in rheumatoid synovial explant tissues and cultured synoviocytes. We previously reported that dex treatment decreased Cox immunostaining in rats injected with mycobacterial adjuvant or SCW (25). Corticosteroids are the most potent endogenous inhibitors of inflammation of RA (33), as well as adjuvant-induced arthritis and SCW-induced arthritis in rats (25, 34). Corticosteroids are the end product of an activated hypothalamic-pituitary-adrenal (HPA) axis. Products of a stimulated immune/inflammatory system, such as IL-1, IL-6, and TNF-α, activate the HPA axis

leading to increased corticosteroid production (35–39). There are data suggesting that patients with RA may have abnormal HPA axis function (40–42). Additionally, RA patients have a lower number of glucocorticoid receptors on peripheral blood lymphocytes (43). Since RA patients express IL-1 in inflamed synovia and may have an inadequate cortisol response to inflammation, our data suggest that high levels of Cox-2 expression could be inappropriately sustained in rheumatoid synovia.

While steroids downregulate many genes involved in the inflammatory response, the inhibition of Cox-2 mRNA may play a significant role in the suppression of inflammation. The mechanism of corticosteroid action in suppression of Cox-2 is not well understood, and may involve the destabilization of Cox-2 mRNA and/or suppression of Cox-2 transcription. Indeed, the Cox-2 transcript contains at least 12 copies of the AUUUA RNA instability motif (44). Whether dex inhibits the Cox-2 mRNA expression in rheumatoid synoviocytes via one or both of these mechanisms is worthy of future study since understanding the mechanism of action of corticosteroids on Cox-2 gene expression may elucidate novel insights into the mechanisms of inflammation and antiinflammatory therapy.

Cox inhibitors are used extensively in the treatment of RA. While these drugs effectively inhibit the acute effects of inflammation and pain, the gastrointestinal and renal side effects in humans limit their usage in high-dose regimens (45). Recent studies have shown that most currently used NSAIDs are able to inhibit the PG synthase activity of both the Cox-1 and -2 isoenzymes (6, 46). However, Meade et al. (46) recently demonstrated that indomethacin inhibited murine Cox-1 at a 22-fold lower concentration than murine Cox-2, whereas 6-methoxy-2-nalpthyl acetic acid (nabumetone) exhibited a preferential inhibitory effect on murine Cox-2. No specific human Cox-2 inhibitors are currently available. These studies suggest that it may be possible to develop selective pharmacological inhibitors of the Cox isoenzymes. Data presented in this study suggest that the Cox-2 isoenzyme could be induced greatly during active inflammation in rheumatoid synovia due to stimulation by IL-1. It is conceivable that NSAIDs that selectively inhibit Cox-2 may exhibit novel therapeutic effects in inflammatory diseases such as RA.

In summary, both Cox-1 and -2 are expressed in rheumatoid synovial explants and cultured rheumatoid synoviocytes under basal culture conditions. However, Cox-2 appears to be the major regulated isoform, exhibiting stimulation by IL-1β or phorbol ester and suppression by corticosteroids. Modulation of Cox-2 in rheumatoid synovial tissues may be an important mechanism regulating inflammation in RA.

Acknowledgments

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