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### **Research Article**

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# Dexamethasone Inhibition of Interleukin 1 Beta Production by Human Monocytes

## Posttranscriptional Mechanisms

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### Abstract

Dexamethasone is known to have an inhibitory effect on IL-1 production. To determine the mechanism(s) of this inhibition, adherent human blood monocytes were stimulated with *Escherichia coli* lipopolysaccharide (LPS) (10 µg/ml) in the presence of dexamethasone. Nuclear transcription run-off assays showed that LPS induced IL-1β gene transcription two- to fourfold and that this induction was unaffected by dexamethasone exposure (10<sup>-5</sup> M). The lack of dexamethasone's transcriptional effects was further supported by the absence of any significant change in IL-1β mRNA accumulation between LPS-stimulated monocytes exposed or unexposed to dexamethasone, as determined by Northern blot analysis.

Posttranscriptionally, dexamethasone was found to have multiple effects: slight prolongation of IL-1β mRNA half-life, moderate inhibition of translation of the IL-1β precursor, and profound inhibition of the release of IL-1β into the extracellular fluid.

The data indicate that IL-1β is first translated as the 33,000-D pro-IL-1β protein, the predominant intracellular form, and the processed to a 17,500-D IL-1β protein before or during extracellular transport. The major inhibitory effects of dexamethasone appear to be directed at the translational and posttranslational steps involved in these events.

### Introduction

IL-1 is a monocyte-macrophage-derived peptide that has potent amplifying effects on inflammatory and immune responses. IL-1 has been reported to raise the thermostat set-point of the hypothalamus (1, 2), induce leukocytosis (3), increase the synthesis of hepatic acute phase protein (4), promote endothelial-leukocyte adhesion (5), induce endothelial cell protein synthesis (6, 7), stimulate fibroblasts (8), and augment T cell proliferation (9). The production of IL-1 by monocytes may be a fundamental event in the initiation and maintenance of inflammatory and immune responses. In turn, the regulation of IL-1 production may prove essential to the ultimate modulation of these reactions.

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There are two species of IL-1, IL-1α and IL-1β (10). Both are 17,000-kD polypeptides that differ in their isoelectric points: IL-1α has a pI of 5.0, whereas IL-1β has a pI of 7.0 (10). Human peripheral blood monocytes and monocyte cell lines, such as THP-1, produce both pI 7.0 (IL-1β) and pI 5.0 (IL-1α) IL-1 in a ratio of 9:1 or greater (10-12). Because both proteins appear to bind to the same receptor (13, 14) and share an identical spectrum of biological activities (15), it seems likely that IL-1β contributes the majority of IL-1 activity derived from human monocytes.

Although numerous soluble and particulate agents are known to trigger the production of IL-1, agents that control the production of this monokine are poorly understood. Recent reports suggest that dexamethasone (16) may be important in IL-1 regulation. Here we report our initial efforts to understand the mechanisms involved in this dexamethasone-mediated effect. Studies with *Escherichia coli* lipopolysaccharide (LPS)-stimulated human monocytes carried out at the protein level, as measured by IL-1 supernatant activity in a thymocyte comitogenic assay, confirmed the inhibition of IL-1 production by dexamethasone. Dissecting the IL-1β production pathway showed that LPS-induced IL-1β gene transcription and IL-1β mRNA accumulation were not affected by exposure of monocytes to dexamethasone. Since these studies suggested posttranscriptional inhibition of IL-1β production, we examined IL-1β mRNA half-life and translation, and elaboration of IL-1β into monocyte supernatants. Posttranscriptionally, dexamethasone had important effects on translation and secretion.

Taken together, these studies point to multiple posttranscriptional IL-1β production steps inhibited by dexamethasone. These data are similar to findings on the control of synthesis of another LPS-induced monokine, cachectin (tumor necrosis factor [TNF])<sup>1</sup> (17), where transcription was somewhat suppressed in the presence of dexamethasone, but inhibition of translation appeared to be the predominant mode of dexamethasone action. Thus, regulation of production of these two monokines in monocytes may occur through similar mechanisms.

### Methods

**Mononuclear cells.** PBMC were obtained by Ficoll-Hypaque centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ) of either venous blood of normal volunteers or leukopheresis byproducts obtained from the Philadelphia Red Cross. Cells at the resulting interface were aspirated, washed three times in HBSS without Ca<sup>++</sup> or Mg<sup>++</sup>, and suspended at a concentration of 5 × 10<sup>6</sup> cells/ml in RPMI 1640 medium.

1. *Abbreviations used in this paper:* rIL-1β, recombinant IL-1β; TNF, tumor necrosis factor.

**Isolation of adherent cells.**  $100\text{--}125 \times 10^6$  PBMC in RPMI with 10% heat-inactivated human serum were incubated in T-150 flasks (Corning Glass Works, Corning, NY) that had been coated with human serum and then incubated for at least 1 h at 37°C in a 5% CO<sub>2</sub> atmosphere. At the end of 1 h, nonadherent cells were gently poured from the flasks and discarded, and the flasks rinsed with RPMI. Adherent cells were released into HBSS by mechanical agitation. The resultant cell suspension was aspirated and centrifuged, and the pellet washed twice in HBSS before resuspension in complete medium for use as adherent cells.

**Sources of IL-1.** IL-1 was generated from adherent cells cultured in RPMI 1640 supplemented with 10% heat-inactivated pooled human sera, using *E. coli* LPS serotype 0111:B4 at 10 µg/ml (Difco Laboratories, Inc., Detroit, MI) as a stimulant (18). The supernatants were recovered by centrifugation at 400 g for 10 min, filtered (0.2 µm), and frozen at -70°C until needed. In those experiments where dexamethasone modulation of IL-1 production was tested, adherent cells were pretreated with dexamethasone for 2 h and then stimulated with LPS. Previous work has shown that 80% of IL-1 activity is released from LPS-stimulated monocytes within 18 h, but no measurable activity is released in the first 2 h (data not shown). Therefore, supernatants used in the IL-1 bioassay were aspirated and discarded after 2 h of culture, and cells were extensively washed to avoid carryover of dexamethasone into the bioassay. The monocytes were recultured with LPS and complete media without dexamethasone, and supernatants were collected over the ensuing 16 h, without loss of measurable IL-1 activity. In immunoprecipitation studies, cell lysates or supernatants were recovered after 18 h without media changes.

**Assay for IL-1.** A standard thymocyte comitogenic assay (19) was performed on the adherent cell supernatants. 8 h before the termination of microtiter cultures, each well was pulsed with 0.5 µCi [<sup>3</sup>H]-thymidine (6.7 Ci/mM sp act; New England Nuclear, Boston, MA) and harvested onto fiberglass filters with a multichannel automated cell harvester (Brawael Biomedical Research and Development Laboratories, Inc., Gaithersburg, MD). Filters were dried, transferred to plastic scintillation vials with 2.0 ml Econofluor (New England Nuclear), and counted in an automated scintillation counter (model LS3801; Beckman Instruments, Inc., Fullerton, CA). Data are reported as mean counts per minute ± standard deviation of triplicate cultures.

**Immunoprecipitation of IL-1β.** In vivo labeling of protein was accomplished by culturing cells in methionine-deficient RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) supplemented with 200 µCi/ml of [<sup>35</sup>S]methionine (800 Ci/mmol sp act; New England Nuclear) and stimulating with LPS. Cells were lysed with 0.05 M Tris (pH 7.4), 0.02% NaAz, 0.5% NP-40, 5 mM EDTA, 10 mM *N*-ethylmaleimide, and 1 mM phenylmethylsulfonyl fluoride for 30 min at 4°C. After lysis, debris was cleared from suspensions by centrifugation at 10,000 g for 30 min. Monocyte supernatants were collected before cell lysis and adjusted to give detergent concentrations identical to lysates. Lysate supernatant samples were cleared by agitating with 5 µl of normal rabbit serum for 15 min followed by 15 min exposure to 15–20 µl of a 10% suspension of Pansorbin (Calbiochem-Behring Corp., La Jolla, CA). The latter had been washed once in 2 mM Tris (pH 7.5), 3% SDS, and 10% 2-mercaptoethanol, heated to 100°C for 10 min, and washed five times in 2 mM Tris (pH 7.5), 0.1% SDS, 0.1% NP-40. Pansorbin was removed by centrifugation (10,000 g, 10 min) and samples transferred to new Eppendorf tubes (Brinkmann Instruments Inc., Westbury, NY). A polyclonal antiserum directed against IL-1β (Cistron, Biotechnology, Pine Brook, NJ; and Dr. P. Simon, Smith Kline & French Laboratories, Swedeland, PA) was added to achieve a final tenfold antibody dilution, and samples were agitated overnight at 4°C. The following day, 0.5 vol of treated Pansorbin was added to the samples and rotated at 4°C for 1 h. Pansorbin beads were recovered by centrifugation (10,000 g, 5 min) and washed five times in 2 mM Tris (pH 7.5), 0.1% SDS, 0.1% NP-40, 0.15 M NaCl. After the final wash, Pansorbin was resuspended in Laemmli buffer (0.08% SDS, 0.7 M 2-mercaptoethanol, 1 M glycerol, 0.06 M Tris [pH 6.8], 0.05% bromophenol blue), heated at 100°C for 10 min, and frozen at -70°C.

SDS-PAGE analysis was performed on immunoprecipitated proteins from lysates and supernatants representing 10<sup>6</sup> cells. A 15% polyacrylamide, 2.7% bis-acrylamide gel was used with a 5% polyacrylamide, 2.7% bis-acrylamide stacking gel. Upper and lower tank buffers consisted of 0.192 M glycine, 0.025 M Tris, 0.1% SDS. Gels were run at a constant current of 15 mA in a vertical slab gel unit (SE-600; Hoefer Scientific Instruments, San Francisco, CA). After electrophoresis, gels were stained with 50% methanol, 10% glacial acetic acid, and 1 mM Coomassie Brilliant Blue R25U, and stain removed with multiple changes of 10% methanol, 10% glacial acetic acid. The gels were soaked for 30 min in Amplify (New England Nuclear), dried for 3 h on a gel slab dryer, and exposed to Kodak XAR-5 film with intensifying screens at -70°C for 24–48 h. Unlabeled recombinant IL-1β used in competition experiments was a generous gift of Dr. P. Simon.

**Preparation and radiolabeling of an IL-1β synthetic oligonucleotide.** A 20 bp (20'-mer) oligonucleotide was manually constructed complementary to base pairs 669–689 of the published human IL-1β gene (pI7) [5'-ACACTCTCCAGCTGTAGAGT-3'] (20) by a solid phase phosphite triester method using diisopropylphosphoramidites (21). All materials were from Applied Biosystems, Inc. (Foster City, CA). Beginning with 1.5 µmol of deoxythymidine-derivatized controlled pore glass (10–15 µm pore size), deoxyribonucleoside-3'-O-phosphoramidites were sequentially added in the usual manner (21). Briefly, dehydrations were performed with 3% wt/vol trichloroacetic acid in dichloromethane. For couplings we used 0.2 M phosphoramidites (30 µmol/cycle) in anhydrous acetonitrile. Couplings were conducted under an argon atmosphere after activation of phosphoramidites with 0.5 M tetrazole. The oxidation step was performed with 0.1 M I<sub>2</sub> in H<sub>2</sub>O/2,6-lutidine/tetrahydrofuran (1:10:40 vol/vol/vol) and capping was accomplished after each cycle with acetic anhydride (acetic anhydride/2,6-lutidine/anhydrous tetrahydrofuran, 1:1:8) mixed 1:1 with 6.5% dimethylaminopyridine in anhydrous tetrahydrofuran for catalysis.

After completion of the synthesis, ~40% of the controlled pore-glass support was treated overnight with 30% NH<sub>4</sub>OH at room temperature followed by a similar treatment at 55°C overnight to remove the oligonucleotide from the solid support and unblock protecting groups. Approximately one-third of the resulting material was then purified. In brief, the completed 20'-mer was purified away from failure sequences by PAGE using a polyacrylamide/bisacrylamide sequencing gel (19:1) containing 8 M urea. The 20'-mer band was visualized by the technique of ultraviolet (UV) shadowing at 254 nm and excised from the gel. The oligonucleotide was then eluted from gel slices in 0.5 M ammonium acetate and further purified by reverse phase exchange chromatography using a C<sub>18</sub> Sep-pak (Waters Associates, Millipore Corp., Milford, MA). The oligonucleotide was eluted in 30% acetonitrile in water, evaporated to dryness, and resuspended in water for storage in -20°C. Approximately 3.0 OD<sub>260 nm</sub> of purified material was obtained.

This 20'-mer was radiolabeled to a specific activity of 2.5–5 × 10<sup>5</sup> cpm/ng using <sup>32</sup>P-gamma-ATP (3,000 Ci/mmol; New England Nuclear) by the T4 polynucleotide kinase method (22) and purified by anion exchange chromatography using DE-52 (Whatman, Inc., Clifton, NJ).

**Northern blot analysis of IL-1β mRNA.** Monocyte stimulation was performed as outlined above. At time points indicated thereafter, total cellular RNA was isolated by a guanidium isothiocyanate method (23) with cesium chloride modification (24). Equal amounts of RNA, determined by A<sub>260 nm</sub>, were denatured at 68°C for 15 min in sample buffer and then size-fractionated by electrophoresis through 1% agarose gel containing 6% formaldehyde and 2 µg/dl ethidium bromide. Agarose gels were visualized by UV illumination to determine position of 28S and 18S rRNA bands, to assess integrity of RNA, and to verify that equal amounts of RNA had been loaded into all wells before transfer to Gene Screen Plus nylon membranes (New England Nuclear). Transfer of RNA to membranes was accomplished by capillary blotting for 24 h using 2.5× SSPE (350 mM NaCl, 80 mM NaH<sub>2</sub>PO<sub>4</sub>, 7 mM EDTA, pH 7.4). After blotting, membranes were dried, baked at 80°C in vacuo, and prehybridized at 55°C for 16–25 h in prehybrid-

ization fluid (10 mM EDTA, 0.5% SDS, 5× Denhardt's solution [1× Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.2% bovine serum albumin], 1% SDS, 1 mM NaCl, 5 mM Tris [pH 7.4]). This was followed by hybridization at 55°C for 24 h in 1 M NaCl, 7 mM EDTA, 0.1 M Tris-HCl (pH 8.0), 5× Denhardt's solution, 0.1% SDS, and 250 µg/ml denatured tRNA containing 2 ng/ml of heat-denatured <sup>32</sup>P-labeled synthetic IL-1β oligonucleotide. The blots were washed in 1 M NaCl, 7 mM EDTA, 0.1 M Tris-HCl (pH 8.0), and 0.5% SDS at 25°C for 10 min, followed by a 1-min wash in the same solution at 55°C. This was repeated four times. Membranes were exposed to Kodak XAR-5 film with Cronex tungstate intensifying screens (DuPont Photo Products, Burbank, CA) at -70°C for 24-72 h.

To assess IL-1β mRNA half-life, 4 h after LPS stimulation actinomycin D (Sigma Chemical Co., St. Louis, MO) was added to cell cultures to achieve a concentration of 10 µg/ml. Total cellular RNA was isolated at time points thereafter.

Autoradiograms were analyzed by laser densitometry at 600 nm, and transmittance of each condition was reported as percent of LPS-induced mRNA accumulation in untreated monocytes.

Radiolabeled probes were removed from blots by incubation with 0.2% SDS, 10 mM Tris at 85°C for 60-90 min. The blots were then exposed to x-ray film to verify that radioactivity had been completely eluted before reprobing.

To ensure equal RNA transfer to membranes, the blots were stripped, prehybridized at 42°C for 24 h in 50% deionized formamide, 1× Denhardt's solution, 1% SDS, 1 mM NaCl, 5 mM Tris (pH 7.4), 0.5 ng/ml heparin, 250 µg/ml of denatured sheared salmon sperm DNA, and 10% dextran sulfate. This was followed by hybridization with prehybridization fluid with 10<sup>6</sup> cpm/lane of a heat-denatured control probe, <sup>32</sup>P-labeled plasmid DNA with a full-length cDNA insert directed against an HLA class I gene (a generous gift from Dr. D. George, University of Pennsylvania) (25) radiolabeled to a specific activity of 10<sup>9</sup> cpm/µg with [<sup>32</sup>P]dCTP using a random primer method (26) and purified by spun column chromatography. Blots were washed twice in 2× standard saline citrate (SSC) (300 mM NaCl, 30 mM sodium citrate), 0.1% SDS at 25°C for 30 min and twice in 0.1× SSC, 0.1% SDS at 50°C for 30 min and exposed to Kodak XAR-5 film as described above.

**Transcriptional run-off assays.** Adherent cells were isolated as outlined above. After 2 h of LPS stimulation or 2 h of dexamethasone pretreatment followed by 2 h of LPS stimulation in the presence of dexamethasone, nuclei were isolated for transcriptional analysis according to the method of Krönke (27). The cells were washed twice in 10 mM Tris (pH 7.5), 10 mM KCl, and 3 mM DTT, followed by lysis in 10 mM Tris (pH 7.5), 2 mM MgCl<sub>2</sub>, 3 mM CaCl<sub>2</sub>, 3 mM DTT, and 0.2% NP-40, with subsequent centrifugation through 2 M sucrose. After resuspension in 100 µl of 50% glycerol, 50 mM Tris (pH 7.5), 3 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, and 0.1 mM EDTA, 10<sup>8</sup> nuclei were frozen in liquid N<sub>2</sub>. When needed, nuclei were thawed on dry ice and 10<sup>8</sup> nuclei added to an equal volume of transcription buffer containing 0.2 M KCl, 4 mM MgCl<sub>2</sub>, 4 mM DTT, 0.8 mM of ATP, CTP, and GTP, and 200 U RNasin (Promega Biotec, Madison, WI). <sup>32</sup>P-Labeled UTP (0.2 mCi, 3,000 Ci/mmol; ICN Radiochemicals, Irvine, CA) was added and the reaction mixture incubated at 26°C for 25 min. Reactions were stopped by two sequential 15-min incubations with 20 µg DNase I (Bethesda Research Laboratories, Gaithersburg, MD) adjusted to 1% SDS and 5 mM EDTA. This was followed by treatment with 1 mg/ml proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, IN) at 42°C for 30 min, and extraction with chloroform/phenol (1:1). The resulting aqueous solutions were centrifuged through a 1-ml Sephadex G-50 column (Pharmacia Fine Chemicals) equilibrated with 1% SDS and 5 mM EDTA, and the <sup>32</sup>P-RNA ethanol-precipitated.

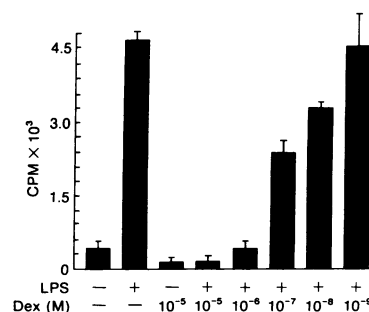
Equal numbers of counts per minute of <sup>32</sup>P-RNAs were then resuspended in 5 ml hybridization buffer (50% formamide, 0.75 M NaCl, 0.5% SDS, 2 mM EDTA, 50 mM Hepes [pH 7.5], 10× Denhardt's solution, 20 µg/ml polyadenylate [Pharmacia Fine Chemicals], and 500 µg/ml denatured salmon sperm DNA [Sigma Chemical Co.]). This was added to T-25 flasks containing prehybridized nitrocellulose filters (0.45 µm; Schleicher & Schuell, Inc., Keene, NH) onto which linear-

ized and denatured IL-1β (generous gift of Dr. U. Gubler of Hoffman-La Roche, Inc., Nutley, NJ) containing the portion of the translated region coding for amino acids 1-139 of the complete IL-1β precursor, pBR322 (negative control) and HLA class I plasmid DNAs equivalent to 0.5 µg of insert DNA (except pBR322 where DNA equivalent to IL-1β plasmid DNA) had been slot blotted using a manifold apparatus (Schleicher & Schuell, Inc.). After hybridization for 3 d, filters were washed three times at 22°C in 2× SSC, 0.1% SDS, and then twice at 50°C in 0.1× SSC and 0.1% SDS, dried, and exposed to Kodak XAR-5 film with intensifying screens at -70°C for 4-7 d.

## Results

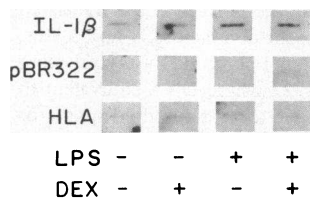
**Dexamethasone inhibits IL-1 activity.** Previous investigators have shown that dexamethasone inhibits IL-1 elaboration into supernatants of dexamethasone-exposed murine monocytes stimulated with LPS (16). As shown in Fig. 1, we confirmed that dexamethasone inhibited production of biologically active IL-1 by human monocytes in a dose-dependent fashion. At high concentrations of dexamethasone (10<sup>-5</sup>-10<sup>-6</sup> M) IL-1 activity was suppressed by 90%. This inhibition declined in a dose-dependent fashion, with no inhibition seen at 10<sup>-9</sup> M. This effect could not be explained by carryover of dexamethasone into the IL-1 bioassay, since addition of exogenous IL-1 to supernatants with low IL-1 activity reconstituted the bioassay response.

**Dexamethasone does not inhibit IL-1β gene transcription.** Having reproduced dexamethasone inhibition of IL-1β production, we then investigated the mechanism(s) of dexamethasone's inhibitory control. Since dexamethasone is thought to regulate the expression of many genes directly at a transcriptional level through its interaction with the glucocorticoid receptor (28), we first examined the effects of dexamethasone on transcription. Transcriptional run-off assays were performed using the method of Krönke (27) with an IL-1β probe obtained from Dr. Gubler. A cDNA probe was used in these studies (see Methods) to maximize binding of all synthesized RNAs and obtain an adequate signal. Previous investigations have shown a rapid induction of the IL-1β gene in a human monocytic leukemia cell line, THP-1, occurring within 2 h (29). Therefore, transcriptional activity was examined 2 h after LPS stimulation. As shown in Fig. 2, LPS exposure resulted in a two- to fourfold increase in IL-1β transcriptional activity over unstimulated cells. The same increase in IL-1β transcriptional activity was seen in LPS-stimulated monocytes even after



**Figure 1.** IL-1 activity in dexamethasone-exposed, LPS-stimulated human monocyte supernatants. 5 × 10<sup>6</sup> normal human monocytes were cultured in RPMI with 10% human serum and exposed to various concentrations of dexamethasone for 2 h, followed by LPS stimulation (10 µg/ml). After 2 h, supernatants were decanted,

cells washed twice with RPMI, and cultured 16 h further in culture media with LPS. Supernatants were filtered (0.2 µm), diluted 1:10, and assayed in a thymocyte comitogenic assay. Results are representative of two experiments.



**Figure 2.** IL-1 $\beta$  transcription run-off assays. After dexamethasone treatment (10<sup>-5</sup> M) and/or LPS stimulation (10  $\mu$ g/ml), monocytes were lysed with NP-40 and their nuclei recovered by centrifugation through a 2 M sucrose gradient. Nuclear

transcription was continued in the presence of [<sup>32</sup>P]UTP. At the end of the incubation period, the reaction was stopped by adding DNase followed by proteinase K, and the mixture extracted with chloroform/phenol (1:1). The aqueous solutions were centrifuged through G-50 columns to remove unincorporated [<sup>32</sup>P]UTP, and [<sup>32</sup>P]RNAs were ethanol-precipitated. Equal numbers of counts were hybridized to nitrocellulose filters onto which linearized and denatured IL-1 $\beta$ , pBR322, and HLA class I plasmid cDNAs had been slot blotted. Equal HLA class I signal ensures identical loading of samples, whereas the pBR322 signal denotes nonspecific RNA binding to plasmid DNA. Results are representative of two experiments.

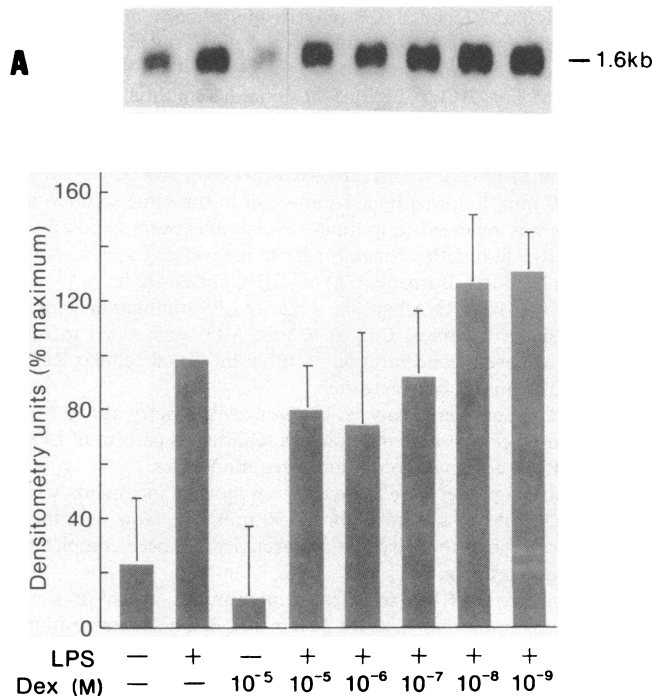
dexamethasone treatment (Fig. 2), suggesting that dexamethasone does not exert inhibitory effects on IL-1 $\beta$  production at a transcriptional level.

These assays also show that low levels of transcriptional activity occurred even in the unstimulated monocytes, and this low level of transcription in unstimulated cells was also not altered by dexamethasone. Whether this level of transcription reflects activation in vivo or during the isolation of monocyte nuclei is unknown.

*Dexamethasone does not inhibit IL-1 $\beta$  mRNA accumulation.* To support data obtained from run-off assays, the effect of dexamethasone on IL-1 $\beta$  mRNA accumulation was assessed by Northern blot analysis. In agreement with others (30), we found that after LPS stimulation, increased IL-1 $\beta$  mRNA accumulation was detectable at 30–60 min, peaked at 2–4 h, and subsequently declined. These events preceded the secretion of maximal levels of biologically active IL-1 in monocyte supernatants by several hours (data not shown). Low levels (5–10% of maximal) of IL-1 $\beta$  transcript were seen in unstimulated, freshly isolated adherent cells, agreeing with the findings in the transcriptional assays (Fig. 2). Again, whether this reflects activation in vivo or during isolation is unknown.

The effects of dexamethasone on IL-1 $\beta$  mRNA accumulation were studied at 4 h, when mRNA accumulation was maximal. Studies performed showed no significant effects (Fig. 3). A weak negative correlation was observed between dexamethasone concentration and IL-1 $\beta$  mRNA accumulation ( $r = -0.555$ ,  $P < 0.001$ ), but comparison of individual experimental conditions (dexamethasone exposed, LPS stimulated) to the positive control (LPS stimulated) was not statistically significant for any dexamethasone concentration. The lack of significant reduction in IL-1 $\beta$  mRNA accumulation further substantiates the lack of transcriptional effects seen with dexamethasone exposure.

*Dexamethasone prolongs IL-1 $\beta$  mRNA half-life.* Major points in posttranscriptional processing were assessed next, with IL-1 $\beta$  mRNA half-life studied first. Monocytes were stimulated with LPS, and when IL-1 $\beta$  mRNA accumulation had reached maximal levels (4 h), actinomycin-D was added to halt further IL-1 $\beta$  production. The rate of IL-1 $\beta$  mRNA degradation was then measured and IL-1 $\beta$  mRNA half-life estimated. The IL-1 $\beta$  mRNA was found to have a half-life of 139 $\pm$ 4 min

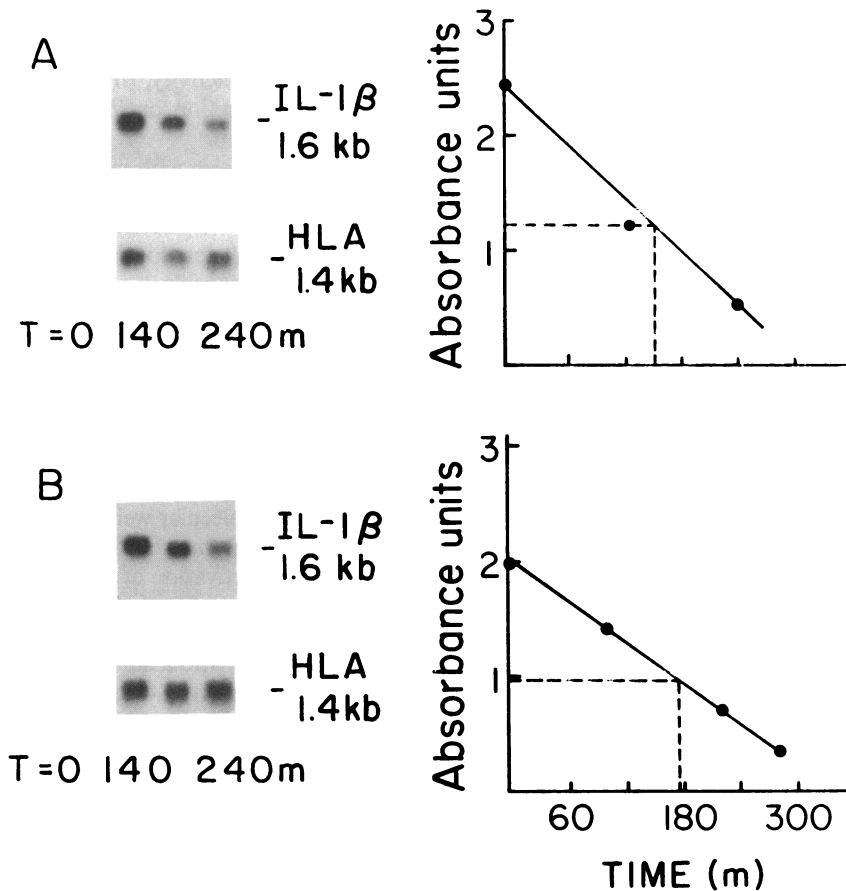


**Figure 3.** Dexamethasone effects on IL-1 $\beta$  mRNA accumulation. Monocytes were stimulated with LPS (10  $\mu$ g/ml) in the presence of various concentrations of dexamethasone. Total cellular RNA was recovered, size fractionated through a 1% agarose gel with formaldehyde, and blotted onto a nylon membrane as outlined in the Methods. Blots were probed with a <sup>32</sup>P-20-mer directed against IL-1 $\beta$  and graphed as density normalized to LPS-stimulated, dexamethasone-unexposed monocyte IL-1 $\beta$  mRNA (A). Equal loading and transfer were ensured by identical UV-induced ethidium bromide fluorescence of ribosomal 18S and 28S bands (B) and equal amounts of HLA class I (control probe) binding between lanes. Results are representative of seven experiments.

(Fig. 4 A) in LPS-stimulated monocytes. Exposure to dexamethasone, followed by LPS stimulation, led to an increase in the IL-1 $\beta$  mRNA half-life to 179 $\pm$ 13 min (Fig. 4 B).

The biological significance of this small increase in IL-1 $\beta$  mRNA half-life is unclear, but indicates that an effect of dexamethasone on IL-1 $\beta$  mRNA cannot explain the observed decrease in IL-1 activity in dexamethasone-exposed, LPS-stimulated monocyte supernatants.

*Dexamethasone inhibits IL-1 $\beta$  translation.* Effects of dexamethasone on IL-1 $\beta$  translation were studied next. Monocytes were cultured in methionine-free medium 1640 supplemented with [<sup>35</sup>S]methionine followed by dexamethasone exposure and/or LPS stimulation. After 18 h, cell supernatants representing all IL-1 $\beta$  production over the entire culture period and lysates representing cytosolic protein at that time were collected, and IL-1 $\beta$  was immunoprecipitated with a rabbit polyclonal antiserum against rIL-1 $\beta$ . SDS-PAGE analysis of biologically radiolabeled immunoprecipitated protein showed that this antiserum recognized a low molecular weight IL-1 $\beta$  species (17.5 kD) and a high molecular weight IL-1 $\beta$  (33 kD)



**Figure 4.** Dexamethasone effects on IL-1 $\beta$  half-life. LPS-stimulated (10  $\mu$ g/ml) (A) and LPS-stimulated, dexamethasone-exposed (10<sup>-5</sup> M) (B) monocytes were cultured for 4 h. Actinomycin D (10  $\mu$ g/ml) was then added to cultures to stop all further RNA synthesis. Total cellular RNA was recovered at time points indicated after adding actinomycin D. Cell viability before RNA recovery was always > 95%. RNA was size fractionated through 1% agarose gels with formaldehyde and blotted onto nylon membranes. Blots were probed with a <sup>32</sup>P-20'-mer directed against IL-1 $\beta$ . Equal loading and transfer were ensured by identical UV-induced ethidium bromide fluorescence of ribosomal 18S and 28S bands and equal amounts of HLA class I (control probe) binding between lanes. Densitometry was performed on the autoradiograms, and area (absorbance units) was plotted vs. time to estimate half-lives. Results are representative of four experiments.

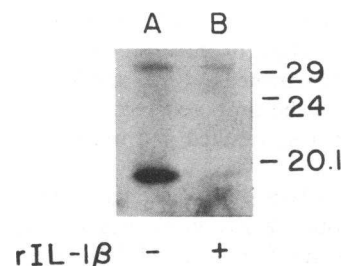
(30, 12) (Fig. 5). Addition of excess unlabeled rIL-1 $\beta$  to both cell lysates and cell supernatants inhibited immunoprecipitation of both high and low molecular weight IL-1 $\beta$  (Fig. 5), providing further evidence of the antiserum's specificity for IL-1 $\beta$ .

In all cell lysates the IL-1 $\beta$  precursor (33 kD) appeared to be the predominant protein form (Fig. 6, lane 4), being at least tenfold more prevalent than the low molecular weight protein.

As shown in Fig. 6, in the cell lysates, dexamethasone reduced the low levels of immunoprecipitable IL-1 $\beta$  in unstimulated monocytes (lane 1) to zero (lane 2) and also reduced by 30–50% the levels of pro-IL-1 $\beta$  (33,000 D) in the stimulated cells (lane 5). The magnitude of this decrease in pro-IL-1 $\beta$

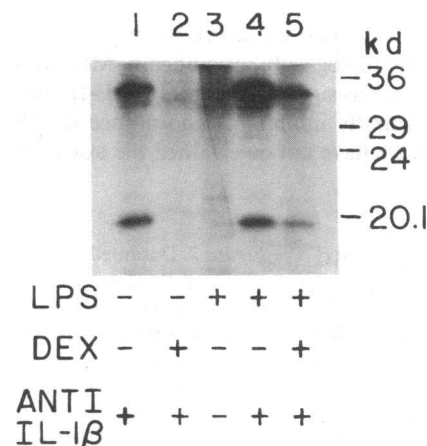
protein synthesis cannot be explained by the slight decreases seen in mRNA accumulation (Fig. 3), but must reflect inhibition of translation itself.

The intracellular low molecular weight IL-1 $\beta$  seen was a



**Figure 5.** Immunoprecipitation of IL-1 $\beta$  by an anti-IL-1 $\beta$  antiserum. Monocytes were cultured in the presence of [<sup>35</sup>S]methionine and stimulated with LPS (10  $\mu$ g/ml). Supernatants were recovered and immunoprecipitated with an anti-IL-1 $\beta$  antiserum and subjected to SDS-PAGE, dried, and autoradiographed at

–70°C for 24 h as outlined in Methods. Both high and low molecular weight IL-1 $\beta$  were immunoprecipitated in LPS-stimulated normal monocytes (A). Unlabeled rIL-1 $\beta$  (500  $\mu$ g) competes with both IL-1 $\beta$  proteins for immunoprecipitation with the antiserum (B).



**Figure 6.** Dexamethasone effects on IL-1 $\beta$  translation. Human monocytes were cultured in the presence of [<sup>35</sup>S]methionine with or without pretreatment by dexamethasone (10<sup>-5</sup> M) and/or LPS stimulation (10  $\mu$ g/ml) as indicated. Immunoprecipitation of IL-1 $\beta$  was carried out with an anti-IL-1 $\beta$  antisera (+) or normal rabbit sera (–) as described in Methods. The lysate from 10<sup>6</sup> cells was subjected to SDS-PAGE, the gels were dried, and autoradiography was performed at –70°C for 24 h. Results are representative of nine experiments.

20,000-D form (lanes 4 and 5). Molecular weight heterogeneity has been reported before (31–33). Dexamethasone exposure often did not decrease the amount of intracellular low molecular weight IL-1 $\beta$  proportionate to the decrease induced in the pro-IL-1 $\beta$  protein. In fact, in some experiments the low molecular weight IL-1 $\beta$  was increased. Processing of the protein from high to low molecular weight form was therefore intact, and an additional inhibitory effect of dexamethasone distal to this step seemed apparent.

*Dexamethasone inhibits IL-1 $\beta$  elaboration.* Supernatants obtained after the entire 18 h culture period were also examined for immunoprecipitable IL-1 $\beta$ . As seen in Fig. 7, abundant low molecular weight IL-1 $\beta$  (17,500 D) was found in monocyte supernatants after LPS exposure, at a 1:5 (33,000:17,500 D) ratio (lane 4). However, none was detected in the supernatants from cells exposed to LPS plus dexamethasone (lane 5). This marked decrease in extracellular IL-1 $\beta$  compared with intracellular precursor further indicates a dexamethasone-induced block in posttranslational events necessary for IL-1 $\beta$  elaboration into supernatants.

## Discussion

The production of IL-1 has been shown to be triggered by many stimuli (34) and regulated by dexamethasone (16) and products of arachidonic acid metabolism (35, 36). Our data show that dexamethasone primarily acts posttranscriptionally. We have previously reported other products that can regulate IL-1 $\beta$  production at a posttranscriptional level, specifically cyclooxygenase inhibitors (37). This has recently been confirmed by Knudsen et al. (38) and shown to be mediated through cAMP.

In this report we have identified events in IL-1 $\beta$  production and found that dexamethasone has multiple effects. At the transcriptional level, the IL-1 $\beta$  gene is induced by LPS, and this induction is unaffected by dexamethasone. Low levels of transcriptional activity are noted in unstimulated cells, and the mechanisms behind this are unknown. Interestingly, this low level of transcription is also unaffected by dexamethasone exposure. Dexamethasone increased IL-1 $\beta$  mRNA half-life, but to a level that is probably biologically insignificant. More importantly, dexamethasone has significant effects on IL-1 $\beta$  protein synthesis and profoundly inhibits its appearance in cell supernatants. Although we have not determined the exact na-

ture of this posttranscriptional mechanism, one can propose several possibilities affecting translation, secretion, or both. Dexamethasone may (i) inhibit tRNA activity, (ii) interfere with protein processing necessary for secretion, or (iii) alter the cell membrane, inhibiting IL-1 $\beta$  secretion.

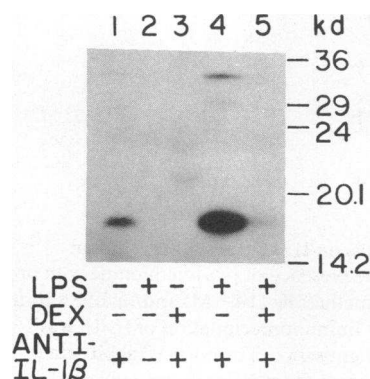
Our studies with the IL-1 $\beta$  protein agree with recent work by Auron et al (12), in that we also found a high molecular weight IL-1 $\beta$  to be the predominant intracellular form, whereas low molecular weight IL-1 $\beta$  is the predominant extracellular form. We also found low concentrations of high molecular weight IL-1 $\beta$  in extracellular fluid, agreeing with other reports of high molecular weight IL-1 $\beta$ -like activity found in stimulated monocyte supernatants (39). The mechanisms involved in elaboration of high molecular weight IL-1 $\beta$  secretion and its biological significance remain unclear.

Our findings disagree with those of Auron et al. concerning cellular low molecular weight IL-1 $\beta$ . Although previous researchers have reported cellular low molecular weight 23,000 D IL-1 $\beta$  (30, 40), Auron et al. suggested that this form of the protein is artifactual owing to poor inactivation of serine proteases, resulting in a degradation product of the cytosolic protein. We found a low molecular weight cellular IL-1 $\beta$  of 20,000 D, a size not predicted by Auron et al., who assumed a trypsin-like cleavage of high molecular weight IL-1 $\beta$ . Also, our protein isolation procedures included use of serine protease inactivators (phenylmethylsulfonyl fluoride) that inhibit proteases capable of degrading high molecular weight IL-1 $\beta$ . Technically, differences are present. Auron et al. obtained cytosol fractions through freeze-thaw cycles, whereas our preparations were obtained by detergent lysis. The 20-kD protein may reflect differences in sample preparation, but most likely represents IL-1 $\beta$  molecular weight heterogeneity as also observed by others (31–33).

Beutler et al. (17) have recently demonstrated that dexamethasone regulates synthesis of cachectin (TNF), another monocyte-derived protein induced by LPS at a translational level more than at a transcriptional level. This report indicates that another functionally important molecule is regulated beyond a transcriptional level in monocytes, and suggests that the entire family of inflammatory mediators may share in this regulatory pathway.

The complete sequence of events that govern the posttranscriptional phase of IL-1 $\beta$  biosynthesis remains unclear. Laput and Beutler (41) identified the presence of a conserved octamer sequence (TTATTTAT) in the 3' untranslated region of mRNAs that specified several endotoxin-inducible inflammatory mediators, including IL-1, cachectin (TNF), lymphotoxin, the interferons, GM-CSF, and fibronectin. They speculated that the sequence may comprise a translational regulatory element involved in the control of these proteins. This possibility has recently been strengthened by Shaw and Kamen (42), who showed that cloning the proposed regulatory octamer into a stable  $\beta$ -globulin gene resulted in a marked decrease in mRNA half-life.

Our data also support recent findings of nontranscriptional effects of glucocorticoids and other steroid hormones (43, 44). Ali and Vedeckis (45) have recently demonstrated tRNA-binding activity of the glucocorticoid receptor and postulate that the activity may reflect posttranscriptional mechanisms of regulating gene expression. They postulate that binding may alter translation efficiencies or modulate protein stability and subsequent degradation. In fact, glucocorticoid therapy has been



**Figure 7.** Dexamethasone effects on IL-1 $\beta$  secretion. Human monocytes were cultured in the presence of [<sup>35</sup>S]methionine after dexamethasone (10<sup>-5</sup> M) and/or LPS (10  $\mu$ g/ml) stimulation as indicated. Immunoprecipitation of IL-1 $\beta$  was carried out with an anti-IL-1 $\beta$  antisera (+) or normal rabbit (-) as described in Methods. The supernatant from 10<sup>6</sup> cells was subjected to SDS-PAGE, the

gels were dried, and autoradiography was performed at -70°C for 24 h. Results are representative of nine experiments.

shown to stimulate muscle wasting associated with protein catabolism (46).

The role of IL-1 $\beta$  in immune and inflammatory states is being increasingly documented (1–9), and dexamethasone is clinically a commonly used immunosuppressive and anti-inflammatory agent. In our experimental system, however, dexamethasone was incapable of significantly inhibiting IL-1 $\beta$  transcription, and some degree of intracellular IL-1 synthesis occurred. Even though dexamethasone interferes with IL-1 $\beta$  translation and its appearance in monocyte supernatants, release of IL-1 $\beta$  upon cell death might still be expected, with resultant immunologic and inflammatory effects. These data may therefore have important implications for future clinical research directed at controlling IL-1-mediated events. Since IL-1 $\beta$  transcription and translation occur even in the face of potent anti-inflammatory agents, development of specific transcriptional inhibitors or receptor antagonists may be necessary to block IL-1 action effectively.

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