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Inhibition by Glucocorticoids of the Formation of Interleukin-1α, Interleukin-1β, and Interleukin-6: Mediation by Decreased mRNA Stability

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SUMMARY

The mechanism by which glucocorticoids inhibit interleukin (IL)-1 and IL-6 formation in human monocytes and a promonocytic cell line activated by *Escherichia coli* lipopolysaccharide was analyzed. Dexamethasone (DEX) decreased levels of IL-1α and IL-1β mRNAs in a dose-related fashion. The DEX-induced decrease in levels of IL-1α and IL-1β mRNAs was abolished by the steroid receptor antagonist RU486. The levels of IL-1α and IL-1β proteins within the cells and of IL-1β in the culture medium were decreased by DEX to comparable extents, so that DEX had no detectable effect on cytokine secretion. DEX did not influence lipopolysaccharide-induced transcription of the IL-1β gene in monocytes. However, DEX markedly decreased the stability of IL-1β mRNA, as shown both by steady state measurements and by pulse-labeling. DEX-induced instability of IL-1β mRNA required protein synthesis. DEX was also found to be a potent inhibitor of IL-1-induced expression of the IL-6 gene in connective tissue-type cells from the synovium of patients with rheumatoid arthritis. Inhibition of the formation of proinflammatory cytokines, including IL-1β and tumor necrosis factor-α, is a mechanism by which glucocorticoids exert anti-inflammatory effects. Inhibition by glucocorticoids of the expression of IL-1α in antigen-presenting cells could decrease the capacity of the cells to stimulate the proliferation of T lymphocytes. This activity, as well as inhibition of the production and effects of IL-1β, including induced formation of IL-6 and of certain lymphokines, could explain the immunosuppressive effects of glucocorticoids.

Cells of the monocyte-macrophage lineage are major producers of TNF-α (1), IL-1 (2), and IL-6 (3). These cytokines have multiple physiological effects, and overproduction could have a serious, and potentially lethal, outcome. When bacteria are injected intravenously into subhuman primates, we have found a sequence of circulating mediators, i.e., first TNF-α, then IL-1β, and finally IL-6, as well as hypotension and shock (4). The hypotension can be reproduced by injection of recombinant IL-1β and is due in part to production of vasodilator prostaglandins. High levels of circulating IL-1 and IL-6 in humans with septicaemia are also associated with a lethal outcome (5, 6). Important feedback regulators of the formation of TNF-α, IL-1, and IL-6 are glucocorticoids. IL-1 acts on the hypothalamus to induce the release of corticotropin-releasing factor (7); corticotropin-releasing factor induces the release by the anterior pituitary of adrenocorticotropic hormone, which in turn stimulates the secretion by the adrenal cortex of glucocorticoids. Glucocorticoids inhibit the formation of TNF-α (1), IL-1β (8), and IL-6 (9).

The effects of glucocorticoids on production of these cytokines are also of interest in relation to the mode of action of synthetic analogs. Although these have long been used as anti-inflammatory and immunosuppressive drugs, their mechanism of action has not been established. Anti-inflammatory effects of glucocorticoids have been attributed to the induction of lipocortins, proteins inhibiting phospholipase A_2 activity (10, 11). However, when lipocortins were cloned and expressed they were found to have sequences identical to those of calpactins, abundant cytoskeletal proteins that are not inducible by glucocorticoids (12, 13). Moreover, lipocortins bind and sequester phospholipids, rather than inhibiting phospholipase activity (14). Thus, the role of lipocortins as mediators of the anti-inflammatory effects of glucocorticoids is in doubt. An alternative explanation for the anti-inflammatory effects of glucocorticoids is that they inhibit the formation of TNF-α and IL-1β, which are proinflammatory (8). Analysis of the molecular biological mechanism by which glucocorticoids inhibit the production of IL-1β is the principal subject of this paper.

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ABBREVIATIONS: TNF, tumor necrosis factor; IL, interleukin; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; GR, glucocorticoid receptor; PMA, phorbol myristate acetate; CHX, cycloheximide; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; GRE, glucocorticoid-response element; DEX, dexamethasone.

A related problem is posed by the existence of two forms of IL-1, namely IL-1α and IL-1β, as opposed to the single forms of most cytokines acting on cells of the immune system (IL-2, IL-3, IL-4, IL-5, IL-6, and IL-7). Both IL-1α and IL-1β bind to common receptors (15) and share many biological activities (2). However, evidence is accumulating that the IL-1α and IL-1β genes are expressed selectively in certain cell types and that the post-transcriptional fates and biological roles of the two molecules are different. For example, the predominant form in monocytes is IL-1β (see below), whereas in keratinocytes it is IL-1α (16). The primary M_r 31,000 product of the IL-1β gene does not bind to the common IL-1α/β receptors and is devoid of biological activity, whereas the primary M_r 31,000 product of the IL-1α gene binds to receptors and is biologically active (17). A proteinase in the cytoplasm of cells of the monocyte lineage cleaves the M_r 31,000 form of IL-1β at a specific site (between Asp^{116} and Ala^{117}) to produce the active M_r 17,500 form, which is secreted (18). Transfected fibroblasts produce the M_r 31,000 form of IL-1β but do not process it and release the biologically active form (18). Observations from our laboratory suggest that IL-1α in the surface membrane of human peripheral blood monocytes and B lymphocytes plays a role in triggering immune responses (19). When these cells are activated with monoclonal antibodies against IL-1α, but not with antibodies against IL-1β or other surface ligands, they stimulate the proliferation of T lymphocytes cultured with them. Thus, IL-1α is active in the antigen-presenting cells in which it is formed. In contrast, IL-1β is secreted and is a component of a cascade of immune responses, of which induction of IL-6 synthesis is an example. To test the hypothesis that glucocorticoids exert immunosuppressive activity by inhibiting the production and effects of IL-1 it is, therefore, necessary to measure the production of IL-1α and IL-1β, as well as a representative mediator induced by IL-1, such as IL-6.

The inhibitory effect of glucocorticoids on IL-1 formation is also of interest from the molecular biological point of view. The mechanism by which glucocorticoids increase the expression of responsive genes has been elegantly analyzed; it is mediated by binding of the GR, in the presence of the hormone or drug, to a DNA enhancer element termed the GRE (20, 21). However, the mechanisms by which glucocorticoids selectively decrease the expression of certain other genes are less well established. Evidence is accumulating for the existence of more than one such mechanism. One is exemplified by a GR-binding region upstream of the prolactin gene (negative GRE) that confers repression by glucocorticoids (22). A second mechanism is post-transcriptional. Thyroid hormones increase the level of HMG-CoA reductase mRNA and protein in liver cells, and glucocorticoids block this effect. Glucocorticoids do not inhibit transcription of the HMG-CoA reductase gene but decrease the stability of the corresponding mRNA (23).

Although it is generally accepted that glucocorticoids inhibit IL-1 formation, as originally proposed by Snyder and Unanue (24), the mechanism by which they do so is controversial. In fact, most of the major possible mechanisms have been postulated, including inhibition of transcription (25), decrease in stability of the IL-1β message (26), and inhibition of secretion (27). In view of the biological importance of the problem, we have examined the effects of DEX on the production of IL-1α, IL-1β, and IL-6 in the human monocytic precursor cell line U937, human peripheral blood monocytes, and connective tissue-type cells from the synovium of patients with rheumatoid arthritis. Effects of DEX on U937 cells were found to be somewhat different from those on monocytes, explaining some of the apparent discrepancies in the literature. Other discrepancies have arisen from the methods used. Western blots and bioassays are at best semiquantitative, and the latter do not discriminate between IL-1α and IL-1β. The specific immunoassays that we have developed for IL-1α, IL-1β, and IL-6 (28, 29) allow the accurate quantification of these proteins, which is required to establish unambiguously the effects of glucocorticoids and other inhibitors of their synthesis.

Materials and Methods

Reagents and cDNA. LPS (*Escherichia coli*, serotype 0111:B4), DEX, PMA, actinomycin D, and CHX were purchased from Sigma Chemical Co. (St. Louis, MO). DEX was dissolved in ethanol (1 mM) and stored at −20°. Human IL-1α and IL-1β cDNAs (30) were obtained from Immunex (Seattle, WA). IL-1α and IL-1β cDNAs were provided by Dr. S. Dower, Immunex Corporation. As previously reported (26), under stringent washing conditions no cross-hybridization occurred. Human IL-6 cDNA (31) was a gift from Dr. P. Seghal (Rockefeller University, New York, NY); β-actin cDNA was a gift from Dr. B. Endlich (University of California, San Francisco, CA); the pBR322 plasmid was purchased from Promega (Madison, WI). RU486 was provided by Roussel-UCLAF.

Cells and culture conditions. Blood was drawn from healthy human volunteers into heparinized syringes, and the mononuclear cell fraction was isolated by centrifugation in Ficoll-Paque (Pharmacia, Piscataway, NJ), prepared in Hanks' balanced salt solution (GIBCO, Grand Island, NY). T lymphocytes were depleted by erythrocyte rosetting. The nonrosetting fraction contained 60–70% nonspecific esterase-positive cells. Nonrosetting cells (6×10^6 cells in 3 ml/well) were preincubated overnight and cultured in RPMI 1640 medium supplemented with 5% human AB serum, at 37° in 5% CO_2/95% air, in six-well plates (Costar, Cambridge, MA). Nonadherent cells were removed by repeated washing in RPMI medium. The residual cells were >85% monocytes, as shown by esterase staining and CD14 positivity.

U937 cells were obtained from the American Type Culture Collection (Rockville, MD). They were cultured as described previously (26), with 3×10^6 cells being used per sample, in six-well tissue culture plates (Costar, Cambridge, MA).

Connective tissue-type cells were recovered by collagenase digestion of synovia from patients with rheumatoid arthritis and were cultured as described previously (9).

RNA isolation and hybridization. RNA for cytoplasmic dot-blots (cytoblots) was prepared as described by White and Bancroft (32). Cytoblot samples were dissolved in 6× standard saline citrate (1× standard saline citrate is 0.15 M NaCl, 0.15 M sodium citrate, pH 7.0) containing 7.5% formaldehyde and were incubated at 60° for 15 min. The samples were applied with suction to a nylon membrane (Nytran, 0.45 μm; Schleicher and Schuell, Keene, NH) supported on a 96-well apparatus (Minifold; Schleicher and Schuell). For Northern blot hybridization, total RNA was prepared in acidified guanidinium thiocyanate and extracted with phenol-chloroform (33). The amount of extracted total RNA was determined by absorbance at 260 nm. RNA samples were subjected to electrophoresis in 1% agarose-2.2 M formaldehyde gels and were transferred to GeneScreen Plus transfer membranes (NEN Research Products, Boston, MA). The prehybridization and hybridization of RNA blots were performed as described previously (26). The extent of hybridization on dot-blots was quantified by measuring ^{32}P radioactivity in a scintillation counter. The counts for hybridized IL-1α, IL-1β, and IL-6 probes were normalized to those for β-actin. The β-actin message level during the experimental period was found to be unchanged if the same amount of RNA was applied for dot-blotting.

IL-1 assays. Culture supernatants and cell lysates of induced and control cells were harvested to measure their IL-1α and IL-1β levels. Cell lysates were prepared by freezing and thawing. IL-1α and IL-1β were assayed in a two-site sandwich ELISA, as described by Kenney *et al.* (28, 29).

Nuclear run-on transcription assays. Isolation of nuclei and nuclear transcription assays were carried out as described by Greenberg and Ziff (34). Each filter was hybridized with the same amount of labeled RNA (10^6 cpm/ml). β-Actin cDNA and pBR322 plasmids were used as controls.

Pulse-chase labeling. Pulse-chase labeling experiments were performed with 20×10^6 cells, as described by Lee *et al.* (26).

Results

Decrease by DEX of levels of IL-1α and IL-1β mRNA in monocytes activated by LPS. To obtain consistent results it was necessary to standardize experimental conditions. In freshly isolated monocytes, and when these cells were cultured in polypropylene tubes, variable amounts of IL-1 mRNAs were observed in the absence of LPS stimulation. However, when the cells were cultured in polystyrene (Costar) plates for 22 hr, background levels of IL-1 mRNA were low and LPS induction of transcription, as well as the effects of DEX, could be consistently observed (Fig. 1A). Northern blot analysis of RNA isolated from LPS-stimulated and DEX-treated cells confirmed dot-blot data (Fig. 1B). DEX was found to decrease steady state levels of both IL-1α and IL-1β mRNA in a dose-dependent manner (Fig. 2). DEX had no effect on the viability of monocytes, as shown by tetrazolium reduction or trypan blue exclusion.

Antagonism of DEX effects in U937 cells by RU486. As reported previously (26), U937 cells activated with PMA and LPS expressed a high level of IL-1β mRNA and a moderate level of IL-1α mRNA (Fig. 3). DEX decreased the levels of both mRNAs (Fig. 3). Preincubation with the steroid receptor antagonist RU486 abrogated the effect of DEX (Fig. 3). Subsequent experiments showed a decrease in levels of IL-1α and IL-1β mRNAs when RU486 and DEX were added at the same time in equimolar concentrations (data not shown). These experiments suggest that the effect of DEX on levels of IL-1α and IL-1β mRNA is mediated through steroid receptors.

Decrease by DEX of the levels of IL-1α and IL-1β in monocytes. When monocytes were cultured in polystyrene plates for 24 hr before LPS stimulation, IL-1α and IL-1β were observed in the cells but not in the culture medium. Because secretion is inhibited under these conditions, effects of DEX on the levels of cell-associated IL-1α and IL-1β reflect total protein synthesis and stability. As shown in Fig. 4, about 6 times as much IL-1β as IL-1α was produced; DEX decreased the amount of IL-1α and IL-1β protein in a dose-dependent manner. This inhibitory effect of DEX was potent; the IC_{50} was of the order of 5 nM, as expected from the affinity of DEX for the GR.

Inhibition by DEX of IL-1β secretion. When monocytes were stimulated with LPS at the time of initiation of culture in polystyrene plates, IL-1β was rapidly formed and secreted and could be measured in 24-hr culture supernatants by immunoassay. As shown in Fig. 5, the addition of DEX at the time of initiation of the culture decreased the amount of IL-1β in the culture supernatants. Even with high concentrations of DEX there was some residual secretion, possibly because of preformed IL-1β. Recently isolated monocytes frequently ex-

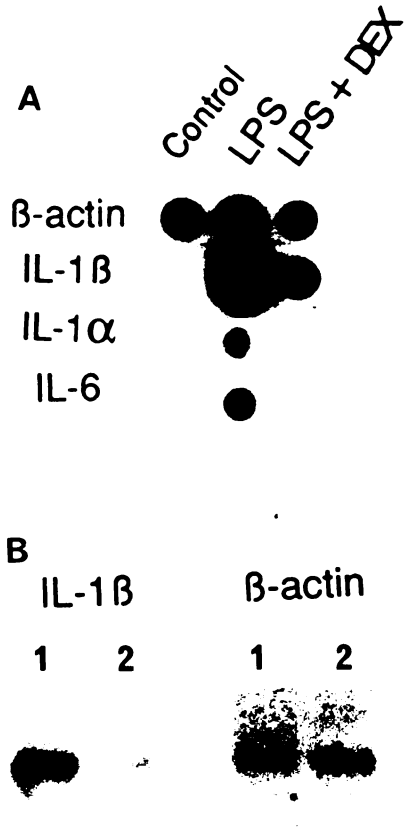

Fig. 1. A, Induction of the expression of IL-1α, IL-1β, and IL-6 mRNA in monocytes by LPS and inhibition by DEX. Cells were preincubated for 22 hr in culture plates before use. Control uninduced cells showed only β-actin mRNA. After induction by LPS (20 μg/ml) for 18 hr, abundant IL-1β mRNA and readily detectable amounts of IL-1α and IL-6 mRNAs were present. After treatment with DEX (10 μM) for 5 hr the amounts of IL-1α, IL-1β, and IL-6 mRNAs were substantially decreased. B, Northern blot analysis of 10 μg of RNA. Monocytes were induced with LPS as in A, in the absence (*lane 1*) and the presence (*lane 2*) of 1 μM DEX. DEX decreased the level of IL-1β mRNA but not of β-actin mRNA.

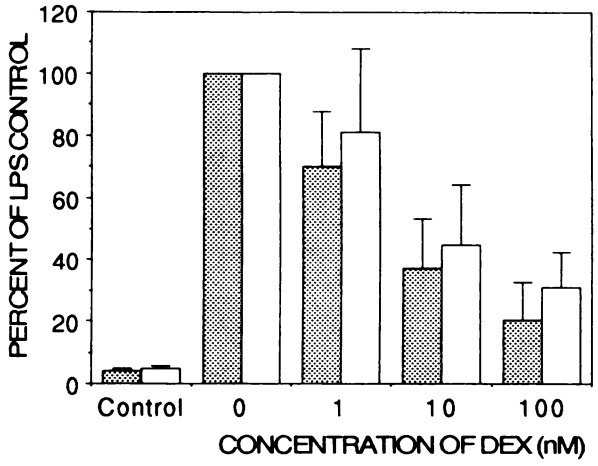

Fig. 2. Dose-dependent inhibitory effect of DEX on steady state levels of IL-1α (□) and IL-1β (□) mRNA. Data were normalized to LPS-treated controls (100%) and are shown as the mean ± standard deviation of nine separate experiments. Experimental conditions were as described for Fig. 1A. Cell viability, as measured by tetrazolium reduction (43), was unaffected by DEX.

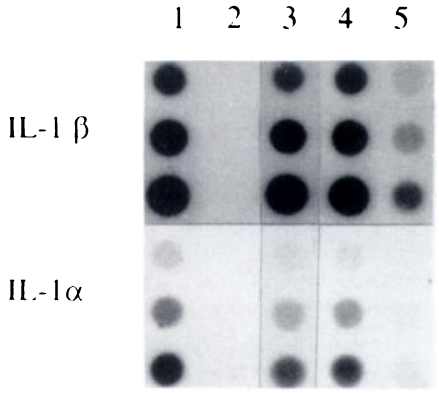

Fig. 3. Effect of the steroid receptor antagonist RU486 on DEX-mediated inhibition of IL-1α and IL-1β mRNA expression. U937 cells were stimulated with 100 nM PMA and 20 μg/ml LPS (*lane 1*). RNA was extracted 18 hr after treatment and high levels of IL-1β and IL-1α mRNAs were detected, compared with the unstimulated control cells (*lane 2*). DEX (100 nM) suppressed IL-1 mRNA expression (*lane 5*). Preincubation with RU486 (10 μM) 2 hr before DEX/PMA/LPS treatment abrogated the DEX effect (*lane 4*). RU486 had no effect on IL-1 mRNA expression in PMA/LPS-stimulated cultures (*lane 3*). β-Actin mRNA levels were similar in all samples (data not shown). Three-fold dilutions of RNA are shown in each panel.

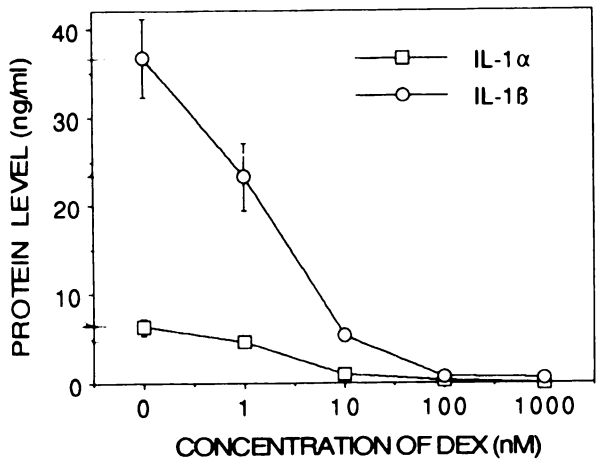

Fig. 4. Dose-dependent effect of DEX on IL-1 protein production in monocyte lysates. Cells were incubated for 24 hr with LPS (20 μg/ml) and with various concentrations of DEX. IL-1β (○) and IL-1α (□) levels in cell lysates were measured by ELISA. The data shown are the mean ± standard deviation of triplicate samples.

press IL-1β mRNA, which could explain some variation in different experiments. However, the dose-response for DEX-mediated reduction of secreted IL-1β (Fig. 5) was comparable to the effect on intracellular IL-1β (Fig. 4) and was certainly not greater. Hence, there was no indication that DEX had a selective effect on IL-1β secretion, as proposed by Kern *et al.* (27).

Lack of effect of DEX on the transcription of the IL-1 gene in monocytes. The experiments described above show that DEX decreases steady state levels of IL-1α and IL-1β mRNA to a degree comparable to effects on the corresponding proteins. To ascertain whether reductions in steady state mRNA levels are due to an effect of DEX on transcription, nuclear run-on experiments were performed with the more abundant IL-1β mRNA (Fig. 6). In nuclei from unstimulated monocytes transcripts of β-actin, but not of IL-1β, were present (data not shown). After LPS stimulation, a relatively high rate

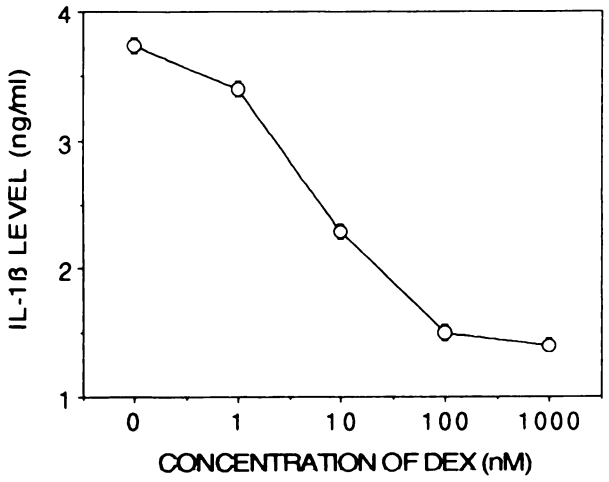

Fig. 5. Dose-dependent inhibition by DEX of IL-1β secretion. Cell culture conditions were as described for Fig. 4. IL-1β levels in culture supernatants were measured by ELISA. The data shown are the mean ± standard deviation of triplicate measurements.

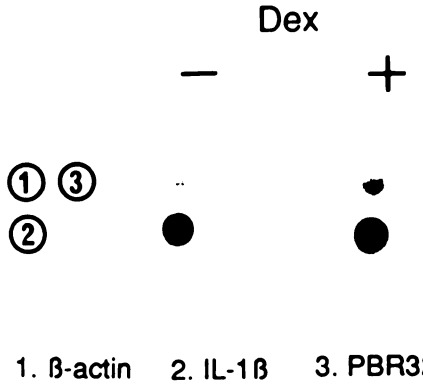

Fig. 6. Effect of DEX on the transcription of the IL-1β gene in monocytes. Cells (2×10^7) were cultured with LPS (20 μg/ml) for 2 hr and for an additional 3 hr in the presence or absence of DEX (10 μM). After incubation cells were lysed and isolated nuclei were incubated with ^{32}P-labeled UTP. Isolated ^{32}P-labeled nuclear RNAs were hybridized to 10 μg of cDNA, which had been immobilized on nitrocellulose filters. Blots were exposed to Kodak XAR film for 7 days.

of transcription of the IL-1β gene, compared with that of the β-actin gene, was found. In nuclei of cells induced with LPS in the presence of even a very high concentration of DEX (10 μM), transcription of the IL-1β gene was not detectably inhibited (Fig. 6).

Decrease of IL-1β mRNA stability in the presence of DEX. The findings described above show that DEX does not inhibit LPS-induced transcription of the IL-1β gene in monocytes but decreases steady state IL-1β mRNA levels. This suggests that DEX may decrease IL-1β mRNA stability. To test that hypothesis, mRNA stability was measured by two independent methods, i.e., the decrease in mRNA levels when transcription was inhibited by actinomycin D and pulse-labeling of IL-1β mRNA with [^{3}H]uridine.

Addition of DEX to monocytes preactivated with LPS, which already had high levels of IL-1β mRNA, led to a rapid decline in IL-1β mRNA levels. When cells were preinduced with LPS and transcription was inhibited by actinomycin D 5 hr later, the level of IL-1β mRNA declined gradually. In cells treated with DEX, IL-1β mRNA levels decreased much more rapidly

(Fig. 7). The addition of DEX together with actinomycin D to induced cells did not lead to a significant acceleration of IL-1β mRNA degradation above that observed in cells treated with actinomycin D in the absence of DEX. If DEX was added in the presence of CHX, superinduction of IL-1β mRNA was observed without accelerated degradation, as in the absence of DEX. Thus, DEX-induced instability of IL-1β mRNA requires both transcription and translation, suggesting that DEX binds to the GR and induces the formation of a protein that selectively targets IL-1β mRNA for destruction.

From this experiment we calculated degradative rates and estimated the half-lives of IL-1β mRNA in the presence of actinomycin D and/or DEX, using accepted calculations (35) (Table 1). Degradation of IL-1β mRNA followed pseudo-first-order kinetics. The IL-1β mRNA fractional degradative rate in actinomycin D-treated monocytes was 0.330 hr^{-1}, whereas after DEX treatment it was 0.113 hr^{-1}. During the same period, the stability of β-actin mRNA was unchanged (data not shown). The findings show that DEX dramatically and selectively accelerates degradation of IL-1β mRNA by a mechanism requiring *de novo* protein synthesis.

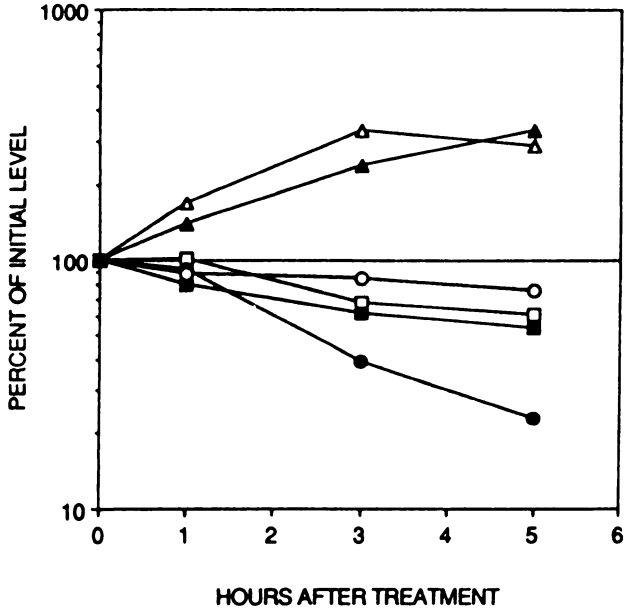

Fig. 7. DEX-induced IL-1β mRNA degradation in monocytes. The steady state level of IL-1β mRNA was analyzed by cytoblot hybridization. Monocytes were induced with 20 μg/ml LPS for 18 hr, at which time DEX (1 μM), actinomycin D (5 μg/ml), CHX (10 μg/ml), or combinations of these were added. At selected times after treatment, RNA was extracted and analyzed. ○, Induced cells; ●, induced cells treated with DEX; □, induced cells treated with actinomycin D; ■, induced cells treated with actinomycin D and DEX; △, induced cells treated with CHX; ▲, induced cells treated with CHX and DEX. Data were plotted as percentage of the initial level before drug treatment. Data shown are the average of three experiments, with standard deviation of ≤20%.

TABLE 1

Measurements of IL-1β mRNA stability

Cells were cultured and treated as described in Figs. 7 and 8. The fractional degradative rate (*K*) was the slope of the linear regression line for the logarithmically transformed data. Half-life, $t_{½}$ = (ln 2/*K*), was calculated from the *K* value.

	Steady state accumulation		Pulse-chase labeling	
	K	Half-life	*K*	Half-life
	hr^{-1}	*hr*	hr^{-1}	*hr*
Control	0.050	13.8	0.047	14.7
DEX	0.330	2.1	0.286	2.4
Act D[a]	0.113	6.1	ND[b]	ND
Act D + DEX	0.125	5.6	ND	ND

[a] Act D, actinomycin D.
[b] ND, not done.

Pulse-chase labeling of IL-1β mRNA. Because the effect of DEX on the stability of IL-1β mRNA was unexpected, an independent method was used to measure mRNA stability; the kinetics of turnover of pulse-labeled mRNA were analyzed. Cells were incubated with LPS in the presence of [^{3}H]uridine, chased with unlabeled uridine, and then treated with DEX. As shown in Fig. 8, DEX significantly accelerated the rate of IL-1β mRNA degradation. The fractional degradative rates of IL-1β mRNA in the absence and presence of DEX were 0.047 hr^{-1} and 0.286 hr^{-1}, respectively (Table 1). The half-life of IL-1β mRNA in DEX-treated cells was calculated from *K* values by two methods; the two values, 2.1 hr from steady state mRNA levels and 2.4 hr from pulse labeling, agree within the limits expected for this type of estimate. In the absence of DEX the estimated half-life of IL-1β mRNA was 13.8 and 14.7 hr, respectively. In both experiments the acceleration by DEX of IL-1β mRNA degradation is unambiguously shown.

Inhibition by DEX of IL-6 formation. In LPS-activated monocytes the IL-6 gene is expressed, and DEX decreased levels of IL-6 mRNA (Fig. 1A). Connective tissue-type cells cultured from synovial tissue of patients with rheumatoid arthritis (synoviocytes) did not express the IL-6 gene unless they were induced with IL-1α or IL-1β, after which a high level of IL-6 mRNA was observed (Fig. 9). DEX significantly inhibited IL-1-induced IL-6 mRNA expression in synoviocytes (Fig. 9).

Discussion

The observations reported in this paper show that DEX decreases levels of IL-1α, IL-1β, and IL-6 mRNAs in LPS-activated monocytes and of IL-6 mRNA in IL-1-activated synoviocytes. The effect on IL-1β was analyzed at the molecular

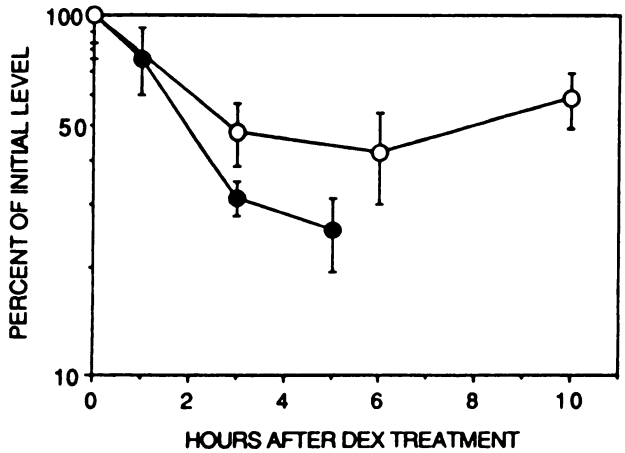

Fig. 8. Analysis of pulse-labeled IL-1β mRNA in monocytes. Cells were pulse-labeled with [^{3}H]uridine for 17 hr, incubated with 5 mM uridine, 2.5 mM cytidine, and 20 μg/ml LPS for 8 hr to deplete the [^{3}H]UTP pool, and washed, and then DEX (1 μM) was added. LPS was present throughout the experiment. At selected time intervals after DEX treatment, RNA was extracted and analyzed. Radioactivity of ^{3}H-labeled IL-1β mRNA on the filter was determined in a liquid scintillation counter and plotted as percentage of initial level at the start of the chase. Each *point* represents the mean ± standard deviation from triplicate counts of each sample. ○, Induced cells; ●, induced cells treated with DEX.

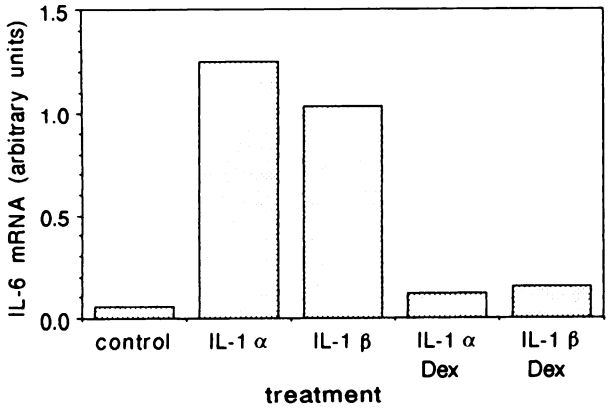

Fig. 9. Induction of IL-6 mRNA expression in connective tissue-type cells from synovial tissue of a patient with rheumatoid arthritis, and inhibition by DEX (1 μM). RNA was extracted from the cells 24 hr after induction with IL-1α or IL-1β (2 ng/ml).

biological level in the human monocyte precursor cell line U937 and in monocytes. The inhibitory effect of DEX was blocked by the steroid receptor antagonist RU486 or by inhibition of protein synthesis. Transcription of the IL-1β gene in LPS-induced monocytes does not require protein synthesis,[2] and nuclear transcription assays showed no inhibitory effect of DEX on IL-1β mRNA or actin mRNA synthesis. In contrast, stability of IL-1β mRNA, measured by two independent methods, was markedly decreased by DEX, from 13.8–14.7 hr to 2.1–2.4 hr. The stability of β-actin mRNA and of c-*fos* mRNA was unaffected by DEX (26), so the effect on IL-1β mRNA stability is selective.

An effect of DEX on mRNA stability is not confined to the IL-1β gene and to monocytes. Administration of DEX to hypophysectomized rats blocked the increase in hepatic HMG-CoA reductase mRNA and enzyme activity that occurred in response to thyroid hormone treatment (23). Nuclear run-on experiments showed no effect of DEX on transcription of the HMG-CoA reductase gene, but DEX decreased the half-life of the corresponding mRNA from 12–15 hr to 2–3 hr. These results suggest that glucocorticoids decrease the abundance of HMG-CoA reductase mRNA by accelerating its degradation. Hence, there are at least two mechanisms by which glucocorticoids decrease expression of genes, the second being mediated by binding of the GR to a negative GRE (22). It will be interesting to define the different situations in which these two mechanisms are operating.

In a wide range of organisms and cell types mRNAs in the cytoplasm differ in stability, and the stability can vary in response to hormonal and other signals (36). Mechanisms by which mRNA is programmed for rapid turnover are being defined. One recognition sequence is the pentamer AUUUA present singly or in multiple reiterations in several oncogene and cytokine 3′ untranslated regions (37). Deletion of this region confers greater stability to messages produced from transfected constructs, and addition of the AU-rich sequences reduces the stability of other messages. A specific protein binding AUUUA-containing RNAs has been identified (38), and it is thought that the formation of this complex may target susceptible mRNA for rapid cytoplasmic degradation. Glucocorticoid treatment does not affect the stability of c-*fos* mRNA (26), so the AU-rich sequence is probably not involved in glucocorticoid-induced instability. However, glucocorticoids could induce the formation of another protein selectively targeting mRNAs for rapid cytoplasmic degradation.

DEX decreases IL-1β mRNA in monocytes without any demonstrable effect on transcription. Knudsen *et al.* (25) reported inhibition by DEX of transcription of the IL-1β gene in U937 cells, and we also found some inhibition of transcription in these cells (26). However, this may be due to inhibition of the PMA-induced priming, by a mechanism dependent on protein synthesis, which is required for efficient transcription of the IL-1β gene in these cells but not in monocytes.[2] In monocytes neither we nor Kern *et al.* (27) found any effect of glucocorticoids on transcription of the IL-1β gene. DEX decreased secreted IL-1β at least as much as intracellular IL-1β, so there was no detectable effect on the secretion of this cytokine, as proposed by Kern *et al.* (27).

Inhibition by glucocorticoids of the formation of IL-1β, as described in this paper, and of TNF-α (1) could account for many of their anti-inflammatory effects (8). The alternative hypothesis, that glucocorticoids induce the formation of lipocortins that inhibit phospholipase A_2 activity, is not supported by recent evidence (12–14).

Processed and secreted IL-1β initiates a cascade of events required for immune responses, including production of IL-2 by T lymphocytic cells (2) and production of IL-6, as described in this paper. IL-6, in turn, is a cofactor for the proliferation of T lymphocytes (39) and for antibody formation by B lymphocytes (3, 40). Hence, inhibition of the formation of IL-1α in antigen-presenting cells, as well as of IL-1β and of IL-6, as described in this paper, could play a major role in the immunosuppressive effects of glucocorticoids. These hormones and drugs also selectively inhibit the formation of some lymphokines, especially IL-4 and IL-5, by the Th2 subset of lymphocytes (8). In contrast, production of the IL-2 receptor by T lymphocytes, and of immunoglobulins by B lymphocytes, is highly resistant to glucocorticoids. Thus, the molecular biological studies now reported provide a rational explanation for the anti-inflammatory and immunosuppressive effects of glucocorticoids.

Examples are given in this paper of glucocorticoids inhibiting the production and effects of IL-1. The converse is also true; low concentrations of IL-1 inhibit the induction by glucocorticoids of phosphoenolpyruvate carboxykinase in the liver (41) and of alkaline phosphatase in endothelial cells (42). In the latter case we found no effect of IL-1 on GR levels. In general, the reciprocal antagonism of IL-1 and glucocorticoids is emerging as a major mechanism regulating responses to infection and inflammation (8).

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