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### Research Article

In bacterial meningitis, LPS induces production in cerebrospinal fluid of the cytokines IL-1 beta and tumor necrosis factor alpha (TNF alpha), which are the principle mediators of meningeal inflammation. IL-1 beta and TNF alpha induce fever, and elevated temperature may affect cytokine expression. Dexamethasone treatment improves outcome in bacterial meningitis possibly by inhibiting IL-1 beta and TNF alpha. In this report, the effects of elevated temperature and dexamethasone on LPS-stimulated IL-1 beta and TNF alpha mRNA gene expression and protein synthesis were studied in human astrocytoma cell lines and primary cultures of human fetal astrocytes. Cells cultured at 40 degrees C exhibited smaller peaks of IL-1 beta and TNF alpha transcription and protein synthesis compared with cells cultured at 37 degrees C. The addition of dexamethasone before, during, or after exposure of the cells to LPS resulted in temperature-dependent inhibition of IL-1 beta transcription and protein synthesis. The most extensive inhibition occurred in pretreated cells cultured at 37 degrees C. Cotreatment with LPS and dexamethasone also inhibited TNF alpha mRNA transcription at both temperatures. The effects of another antiinflammatory agent, indomethacin, on LPS induction of IL-1 beta and TNF alpha mRNA were temperature and cell line dependent. These findings provide a possible explanation for the efficacy of dexamethasone treatment of bacterial meningitis and support the proposal that fever may be [...]

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# Temperature-dependent Modulation of Lipopolysaccharide-induced Interleukin-1 $\beta$ and Tumor Necrosis Factor $\alpha$ Expression in Cultured Human Astroglial Cells by Dexamethasone and Indomethacin

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## Abstract

In bacterial meningitis, LPS induces production in cerebrospinal fluid of the cytokines IL-1 $\beta$  and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), which are the principle mediators of meningeal inflammation. IL-1 $\beta$  and TNF $\alpha$  induce fever, and elevated temperature may affect cytokine expression. Dexamethasone treatment improves outcome in bacterial meningitis possibly by inhibiting IL-1 $\beta$  and TNF $\alpha$ . In this report, the effects of elevated temperature and dexamethasone on LPS-stimulated IL-1 $\beta$  and TNF $\alpha$  mRNA gene expression and protein synthesis were studied in human astrocytoma cell lines and primary cultures of human fetal astrocytes. Cells cultured at 40°C exhibited smaller peaks of IL-1 $\beta$  and TNF $\alpha$  transcription and protein synthesis compared with cells cultured at 37°C. The addition of dexamethasone before, during, or after exposure of the cells to LPS resulted in temperature-dependent inhibition of IL-1 $\beta$  transcription and protein synthesis. The most extensive inhibition occurred in pretreated cells cultured at 37°C. Cotreatment with LPS and dexamethasone also inhibited TNF $\alpha$  mRNA transcription at both temperatures. The effects of another antiinflammatory agent, indomethacin, on LPS induction of IL-1 $\beta$  and TNF $\alpha$  mRNA were temperature and cell line dependent. These findings provide a possible explanation for the efficacy of dexamethasone treatment of bacterial meningitis and support the proposal that fever may be beneficial to the host in this disease. (*J. Clin. Invest.* 1991. 87:1674–1680.) Key words: cytokines • endotoxin • inflammation • astrocytes • fever

## Introduction

Adverse outcome from bacterial meningitis is, for the most part, a result of the host's exaggerated inflammatory response. The cytokines, IL-1 $\beta$  (1) and tumor necrosis factor  $\alpha$  (cachectin) (TNF $\alpha$ )<sup>1</sup> (2) are responsible for a variety of host acute in-

flammatory responses. Both cytokines are primarily synthesized by monocytes and macrophages in response to multiple stimuli, and are also produced in other activated cells including astrocytes and microglial cells (1, 2). IL-1 $\beta$  and TNF $\alpha$  induce fever, stimulate vascular endothelial cells to induce adhesion and passage of neutrophils into the central nervous system (CNS) and other tissues, and trigger inflammatory processes (3, 4).

TNF $\alpha$  and IL-1 $\beta$  act synergistically in the initiation of meningeal inflammation (5, 6). In the rabbit meningitis model, IL-1 $\beta$  and TNF $\alpha$  concentrations in cerebrospinal fluid (CSF) rose sharply after intracisternal injection of purified recombinant *Haemophilus influenzae* lipooligosaccharide into the CSF (7, 8). Furthermore, intracisternal injection of recombinant rabbit IL-1 $\beta$  (rrIL-1 $\beta$ ) or of natural rabbit TNF $\alpha$  initiated the meningeal inflammatory cascade in this animal model and that response was abated by simultaneous administration of monoclonal anti-rabbit IL-1 $\beta$  antibody or of anti-rabbit TNF $\alpha$  antibody (6). Similarly, simultaneous intracisternal administration of anti-rabbit TNF $\alpha$  antibody and anti-rrIL-1 $\beta$  antibodies with *H. influenzae* lipooligosaccharide substantially attenuated the meningeal inflammatory response (6). Elevated concentrations of IL-1 $\beta$  and TNF $\alpha$  in CSF were detected in patients with bacterial meningitis and those concentrations correlated significantly with the amount of CNS inflammation and with outcome from disease (9–11).

Dexamethasone therapy reduced the concentrations of IL-1 $\beta$  and TNF $\alpha$  in CSF of patients with bacterial meningitis (10). Furthermore, clinical studies have demonstrated that dexamethasone therapy diminished significantly the CNS inflammatory response, lumbar CSF pressure, and the incidence of neurological sequelae including bilateral moderate or greater sensorineural hearing loss resulting from bacterial meningitis (12, 13). These findings suggest that the protective effect of dexamethasone in bacterial meningitis may be a result of the suppression of IL-1 $\beta$  and TNF $\alpha$  in the central nervous system.

In this report, cell culture systems were used to investigate cellular and molecular aspects of the induction of IL-1 $\beta$  and TNF $\alpha$  by LPS in human astroglial cells, and to study the effects of temperature, dexamethasone, and indomethacin on this process.

## Methods

**Tissue culture.** Human fetal astrocytes were obtained from 16–18-wk abortuses as part of a protocol approved by the Human Research Review Committee of the University of Texas Southwestern Medical Center. Fetal astrocytes were purified by finely mincing brain tissue into 1-mm pieces, desiccating cells for 30 min to promote attachment, and culturing at 37°C in 5% CO<sub>2</sub> in a 4:1 mixture of DME: medium

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1. Abbreviations used in this paper: CSF, cerebrospinal fluid; GADPH, human glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; TNF $\alpha$ , tumor necrosis factor alpha.

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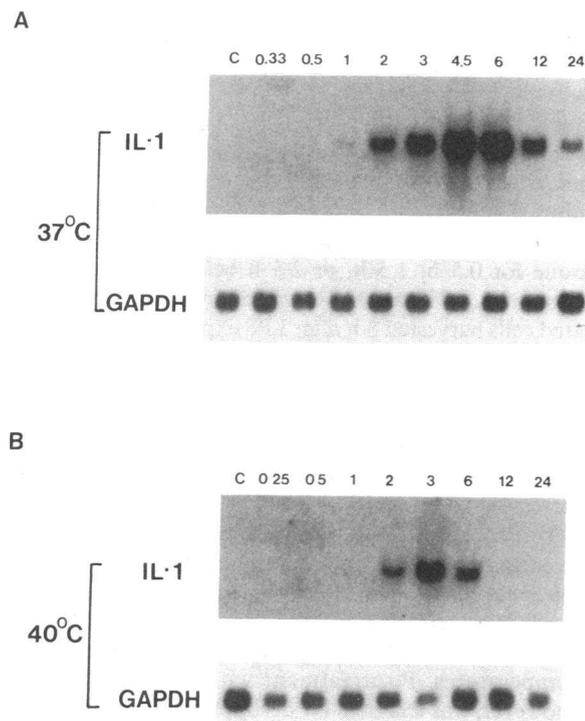
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199 (Gibco Laboratories, Grand Island, NY) containing 10% defined supplemental bovine serum, antibiotics, and L-glutamine. After the first passage, 99% of the cells were immunohistochemically positive for glial fibrillary acidic protein (GFAP). Fetal astrocytes were also transformed by electroporation with a pSV3neo vector (obtained from American Type Culture Collection [ATCC, Rockville, MD]) containing SV40 T antigen and the dominant selectable neomycin gene (14, 15). The human astrocytoma cell lines U-87 and U-373 were obtained from ATCC. Cell lines were cultured at 37°C or 40°C in 5% CO<sub>2</sub> in RPMI 1640 medium (Gibco) supplemented with 10% calf serum, antibiotics, and L-glutamine. IL-1 $\beta$  and TNF $\alpha$  were induced by treating cells at 80% confluence with concentrations of 500 ng/ml to 5  $\mu$ g/ml LPS (*Escherichia coli* serotype 0127:88, Sigma Chemical Co., St. Louis, MO). Cells were also treated with 10<sup>-5</sup> M to 10<sup>-8</sup> M dexamethasone, and 5  $\mu$ g/ml indomethacin. Treated cells were harvested by trypsinization and cell pellets were stored at -70°C for subsequent molecular studies.

**IL-1 $\beta$  assay.** Cell pellets and supernatants were collected from LPS-stimulated cells. With the astrocytoma cell lines and pSV3neo transformed fetal astrocytes, 1  $\times$  10<sup>6</sup> cells per time point were used. With primary cultures of fetal astrocytes, 2  $\times$  10<sup>4</sup> cells per time point were used. Cell lysates were obtained by repeated freezing and thawing of the cell pellets. 100- $\mu$ l aliquots of cell lysates and supernatants were assayed for IL-1 $\beta$  by ELISA (Cistron Technologies, Pine Brook, NJ) as described previously (7, 10). The lower limit of detectable IL-1 $\beta$  in this assay is 20 pg/ml.



**Figure 1.** LPS induction of IL-1 $\beta$  mRNA at 37°C and 40°C. U-373 cells were treated with LPS 500 ng/ml and harvested at the times indicated. The numbers refer to the incubation time in hours. Cells were cultured at 37°C (A) or 40°C (B). 20  $\mu$ g of total cellular RNA from cells at each time point was separated by gel electrophoresis, and transferred to a nylon membrane. The nylon membrane was hybridized first to a <sup>32</sup>P-labelled human IL-1 $\beta$  cDNA probe and subsequently to a GAPDH probe. GAPDH is a constitutively expressed (housekeeping) gene that is expressed at constant levels in all cells and serves as an internal control for the amount of RNA loaded in each lane.

**TNF $\alpha$  assay.** Cell pellets, lysates, and supernatants were collected as described for the IL-1 $\beta$  assay. TNF $\alpha$  protein was assayed in 100- $\mu$ l aliquots using an ELISA kit (T Cell Sciences) according to the conditions recommended by the supplier. Optical density measurements were made with a computerized automated ELISA reader (Bio-rad Laboratories, Richmond, CA) with a standard curve range of 0–1,000 pg/ml. The lower limit of detectability was 10 pg/ml.

**Molecular studies.** Extraction of total cellular RNA with guanidinium isocyanate, denaturing gel electrophoresis, Northern blotting to Nytran filters (Schleicher and Schuell, Keene, NH), random priming, and hybridization conditions were as described previously (16). The IL-1 $\beta$  probe is a full-length human cDNA clone (17). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a constitutive (“housekeeping”) gene that is expressed at constant levels in all cells and served as an internal control for the amount of RNA loaded in each lane. The IL-1 $\beta$ , IL-6, and GAPDH clones were obtained from ATCC. The human TNF $\alpha$  probe is a full-length cDNA clone that was obtained from B. Beutler (University of Texas Southwestern Medical Center at Dallas) (18).

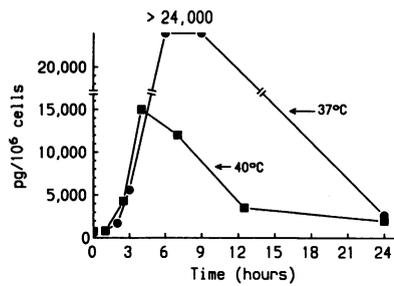
## Results

**Kinetics of LPS induction of IL-1 $\beta$  at 37°C and 40°C.** Of nine different astrocytoma cell lines tested for their ability to produce cytokines in response to LPS (data not shown), the U-373 cell line was chosen for further investigation because it expressed the astrocyte differentiation marker GFAP and demonstrated the most reproducible results.

While it has been observed that IL-1 $\beta$  (and TNF $\alpha$ , see below) induce fever, it has also been suggested that elevated temperatures affect cytokine expression (19). To determine the effect of elevated temperature on LPS induction of IL-1 $\beta$  expression, the kinetics of IL-1 $\beta$  mRNA expression in the U-373 astrocytoma cell line were measured at 37°C and 40°C by Northern blot hybridization analysis. Cells were exposed to LPS 500 ng/ml, and RNA was extracted from cells at multiple subsequent time points. At 37°C, IL-1 $\beta$  mRNA was initially detected 1 h after treatment with LPS, peaked between 4.5 and 6 h, and was minimally detectable at 24 h (Fig. 1 A). At 40°C, IL-1 $\beta$  mRNA was initially detected at 2 h, peaked at 3 h, and was no longer detectable at 12 h (Fig. 1 B). Overall, the amount of IL-1 $\beta$  mRNA in cells cultured at 40°C was substantially reduced compared to cells cultured at 37°C. Expression of a constitutive (housekeeping) gene, GAPDH, was the same at both temperatures. IL-1 $\beta$  (and TNF $\alpha$ , see below) mRNA induction by LPS was also dependent on LPS concentration. The maximal stimulatory effect was observed at 500 ng/ml, whereas higher concentrations were less effective (data not shown).

IL-1 $\beta$  protein was initially detected at 3 h in cells cultured at 37°C or 40°C. Peak synthesis occurred between 5 and 9 h at 37°C and 4 h at 40°C. Substantially more IL-1 $\beta$  protein was synthesized in cells cultured at 37°C than at 40°C (Fig. 2).

**Kinetics of LPS induction of TNF $\alpha$  at 37°C and 40°C.** The induction of TNF $\alpha$  mRNA by LPS at 37°C and 40°C in U-373 cells was similarly investigated by Northern blot hybridization analysis. Overall, TNF $\alpha$  mRNA expression was substantially lower than IL-1 $\beta$  mRNA expression. Autoradiographs of Northern blot hybridization experiments were exposed for 1 wk to detect TNF $\alpha$  signal compared to 24-h exposure for IL-1 $\beta$ . At 37°C, TNF $\alpha$  mRNA was first detectable at 2 h, peaked at 3 h, and was no longer detectable at 12 h (Fig. 3 A). At 40°C, lower amounts of TNF $\alpha$  mRNA were measurable at 2 h and at 3 h and were not detectable at the other time points (Fig. 3 B).

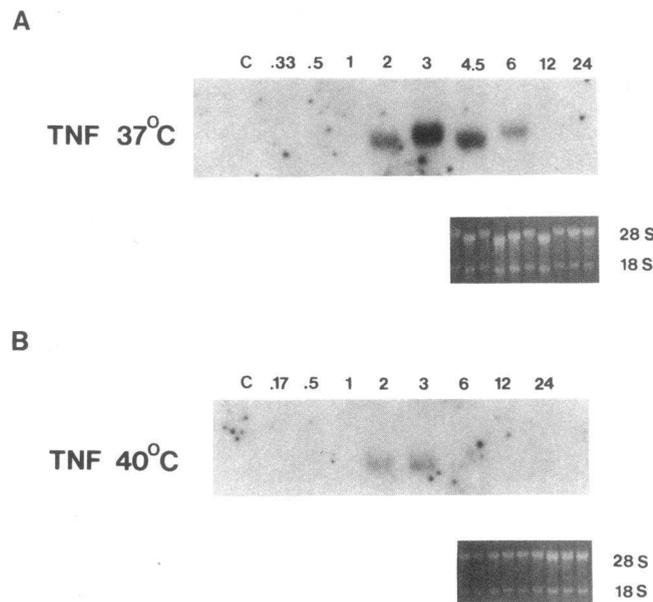


**Figure 2.** LPS induction of IL-1 $\beta$  protein in U-373 cells. U-373 cells cultured at 37°C (circles) or 40°C (squares) were treated with LPS 500 ng/ml and harvested at the indicated time points. IL-1 $\beta$  protein (in picograms per 10<sup>6</sup> cells) was measured in cell extracts as described in Methods. 1  $\times$  10<sup>6</sup> cells were used for each experiment. 24,000 pg/10<sup>6</sup> cells was the upper limit of detection in this experiment. The concentration of IL-1 $\beta$  protein at 37°C from 3.5 to 9 h exceeded the limits of detection. The values at these time points are depicted as half-circles.

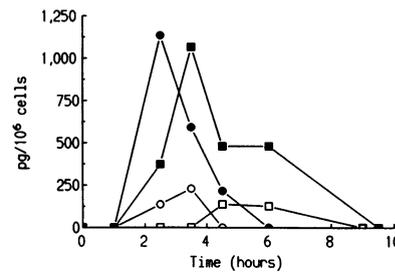
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TNF $\alpha$  protein was measured in LPS-treated U-373 cells at 37°C with peak concentrations observed in cell pellets at 2.5 h and in supernatants at 3.5 h (Fig. 4). At 40°C, TNF $\alpha$  protein was markedly lower in cell pellets and supernatants compared to cells cultured at 37°C (Fig. 4).

*Effects of dexamethasone on LPS-induced IL-1 $\beta$  expression.* The results from animal studies and clinical trials



**Figure 3.** LPS induction of TNF $\alpha$  mRNA at 37°C and 40°C. In a parallel experiment to the one depicted in Fig. 1, U-373 cells were treated with LPS 500 ng/ml and harvested at the times indicated. The numbers refer to the incubation time in hours. Cells were cultured at 37°C (A) or 40°C (B). 20  $\mu$ g of total cellular RNA from cells at each time point was separated by gel electrophoresis, and transferred to a nylon membrane. The nylon membrane was hybridized to a <sup>32</sup>P-labelled human TNF $\alpha$  probe. Because of the risk of losing RNA by stripping the membranes, duplicate membranes containing equal amounts of RNA were used for the experiments depicted here and in Fig. 1. In this case, a photograph of the ultraviolet light-illuminated, ethidium bromide-stained gel depicting the signal intensity of the 28S and 18S rRNA bands demonstrates that equal amounts of RNA were loaded in each lane.



**Figure 4.** LPS induction of TNF $\alpha$  protein in U-373 cells. U-373 cells cultured at 37°C (closed circles and squares) or 40°C (open circles and squares) were treated with LPS 500 ng/ml and cell extracts (circles) and supernatants (squares) were harvested

at the indicated time points. TNF $\alpha$  protein (in picograms per 10<sup>6</sup> cells) was measured by ELISA as described in Methods. 1  $\times$  10<sup>6</sup> cells were used for each experiment.

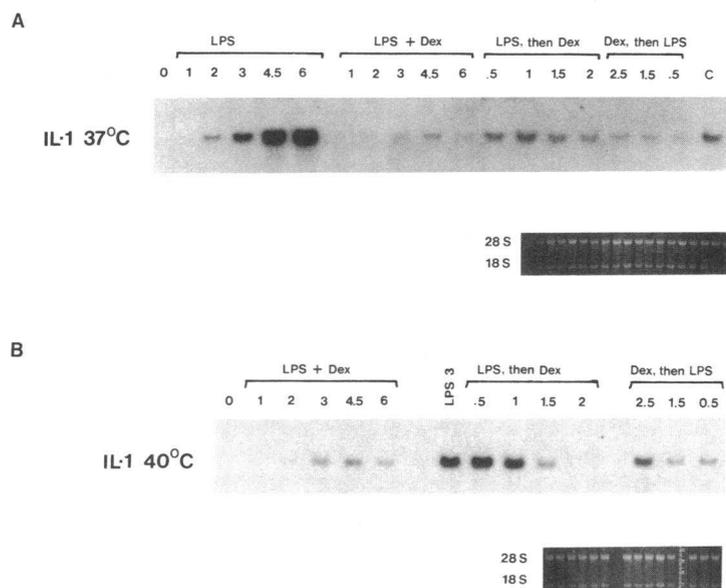
prompted the hypothesis that the protective effect of dexamethasone therapy in bacterial meningitis was a result of the inhibition of IL-1 $\beta$  and TNF $\alpha$  expression. This hypothesis was tested in our system by simultaneously treating cells with LPS and dexamethasone. Induction of IL-1 $\beta$  mRNA by LPS was extensively inhibited by dexamethasone at all time points tested (Fig. 5 A). To determine if the effect of dexamethasone was dose dependent, U-373 (and U-87 cells, see below) were simultaneously treated with LPS and concentrations of dexamethasone ranging from 10<sup>-5</sup> to 10<sup>-8</sup> M. Dexamethasone inhibited IL-1 $\beta$  mRNA induction at all concentrations tested, but was maximally effective at 10<sup>-5</sup> M for U-373 cells and 10<sup>-7</sup> M for U-87 cells (Fig. 7 D). In the absence of LPS, dexamethasone treatment did not stimulate IL-1 $\beta$  or TNF $\alpha$  mRNA expression (data not shown).

To investigate the effects of timing of administration of dexamethasone in relation to LPS treatment in vitro, U-373 cells cultured at 37°C were treated with dexamethasone before and after exposure to LPS. Cells were pretreated with dexamethasone for 0.5 h, 1.5 h, or 2.5 h before LPS challenge. Expression of IL-1 $\beta$  in mRNA extracted from dexamethasone pretreated cells harvested 3 h after LPS exposure is depicted in Fig. 5 A. Maximal suppression of IL-1 $\beta$  mRNA occurred in cells pretreated with dexamethasone for 0.5 h. Cells were also posttreated with dexamethasone after exposure to LPS. Cells were exposed to LPS for 3 h, treated with dexamethasone, and harvested at 0.5 h, 1 h, 1.5 h, or 2 h. While there was substantial inhibition of IL-1 $\beta$ , it was not as pronounced as in cells simultaneously treated or pretreated with dexamethasone (Fig. 5 A).

The effects of elevated temperature (40°C) on dexamethasone suppression of LPS-induced IL-1 $\beta$  mRNA expression in U-373 cells were variable (Fig. 5 B). Simultaneous exposure to LPS and dexamethasone equally suppressed IL-1 $\beta$  mRNA at both temperatures. Posttreatment with dexamethasone at 40°C, however, was less effective at suppressing IL-1 $\beta$  after 0.5 and 1.5 h, but was more effective at 1.5 and 2 h compared to cells cultured at 37°C. Pretreatment with dexamethasone was slightly less effective at 40°C than at 37°C for all time points tested.

The effects of dexamethasone on LPS-induced IL-1 $\beta$  protein synthesis were also studied. Dexamethasone correspondingly inhibited IL-1 $\beta$  protein synthesis in LPS-stimulated U-373 cells (Fig. 6).

To confirm that these observations were not unique to the particular cell line tested, a second astrocytoma cell line, U-87, was similarly investigated for dexamethasone suppression of



**Figure 5.** Dexamethasone suppression of IL-1 $\beta$  mRNA induction by LPS at 37°C and 40°C in U-373 cells. U-373 cells were cultured at 37°C (A) or 40°C (B). IL-1 $\beta$  mRNA expression was measured by Northern blot analysis as outlined in Methods and in Fig. 1. Brackets distinguish lanes containing RNA from cells treated as follows: LPS refers to cells that were treated with LPS alone and harvested at the time points (in hours) indicated by the numbers below the bracket; LPS + Dex refers to cells that were simultaneously treated with LPS and dexamethasone ( $10^{-5}$  M) with cells harvested at the indicated time points (numbers under bracket); LPS, then Dex refers to cells that were first treated with LPS for 3 h and then exposed to dexamethasone with cells harvested at the indicated time points; Dex, then LPS refers to cells that were pretreated with dexamethasone and then exposed to LPS for 3 h with cells harvested at the times indicated below the bracket. C corresponds to cells treated with LPS alone for 3 h at 37°C. LPS 3 indicates the equivalent control for cells cultured at 40°C. In this case, a photograph of the ultraviolet light-illuminated, ethidium bromide-stained gel depicting the signal intensity of the 28S and 18S rRNA bands demonstrates that equal amounts of RNA were loaded in each lane.

LPS-induced IL-1 $\beta$  mRNA expression. The kinetics of LPS induction of IL-1 $\beta$  mRNA in U-87 cells differed from the U-373 cell line. In U-87 cells, IL-1 $\beta$  mRNA was detectable within 15 min of exposure to LPS 5  $\mu$ g/ml with a peak at 30 min. A second peak occurred at 3 h that was sustained for at least 24 h (Fig. 7 A). Simultaneous treatment of U-87 cells with LPS and dexamethasone resulted in complete inhibition of IL-1 $\beta$  mRNA expression within 3 h (Fig. 7 A). The optimal inhibitory concentration of dexamethasone was  $10^{-7}$  M (Fig. 7 D).

U-87 cells were similarly treated with dexamethasone before and after exposure to LPS. Cells were pretreated with dexamethasone 0.5 or 1 h before LPS challenge. Expression of IL-1 $\beta$  mRNA in mRNA extracted from cells harvested 0.5 and 1 h after LPS exposure is depicted in Fig. 7 B. Cells were also posttreated with dexamethasone at 0.5 h after exposure to LPS (Fig. 7 C). The most dramatic inhibition of IL-1 $\beta$  mRNA occurred when cells were pretreated 1 h before exposure to LPS. However, posttreatment with dexamethasone at both 0.5 and 1 h also substantially inhibited IL-1 $\beta$  mRNA within 15 min.

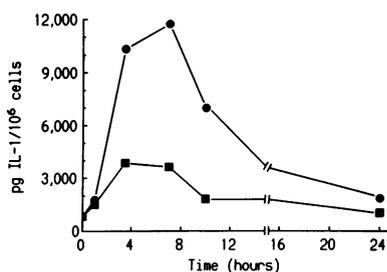
**Effects of dexamethasone on LPS induction of IL-1 $\beta$  and TNF $\alpha$  in primary astrocytes.** To substantiate that our observations were not limited to tumor cell lines, primary cultures of human fetal astrocytes were similarly tested for the ability of LPS to induce (and dexamethasone to suppress) IL-1 $\beta$  and TNF $\alpha$  synthesis (Fig. 8). LPS induced both IL-1 $\beta$  and TNF $\alpha$  protein synthesis in fetal astrocytes (Fig. 8 A), and induced IL-1 $\beta$ , but not TNF $\alpha$ , in fetal astrocytes transfected SV40 T antigen (Fig. 8 B). Cotreatment with dexamethasone moderately suppressed IL-1 $\beta$  and completely suppressed TNF $\alpha$  induction in primary astrocytes (Fig. 8 A). Dexamethasone also suppressed LPS-induced IL-1 $\beta$  in SV40 T antigen transfected astrocytes at 2 and 3 h, but not at 6 h (Fig. 8 B).

**Effects of dexamethasone on LPS-induced TNF $\alpha$  expression.** Dexamethasone suppressed LPS induction of TNF $\alpha$  mRNA in U-373 cells at concentrations of dexamethasone ranging from  $10^{-5}$  M to  $10^{-8}$  M (Fig. 9 A). There was no significant difference in suppression if cells were pretreated, post-treated, or incubated at 40°C (data not shown). The suppressive effects of dexamethasone on TNF $\alpha$  expression in U-87

cells could not be studied because LPS does not induce detectable amounts of TNF $\alpha$  mRNA in these cells.

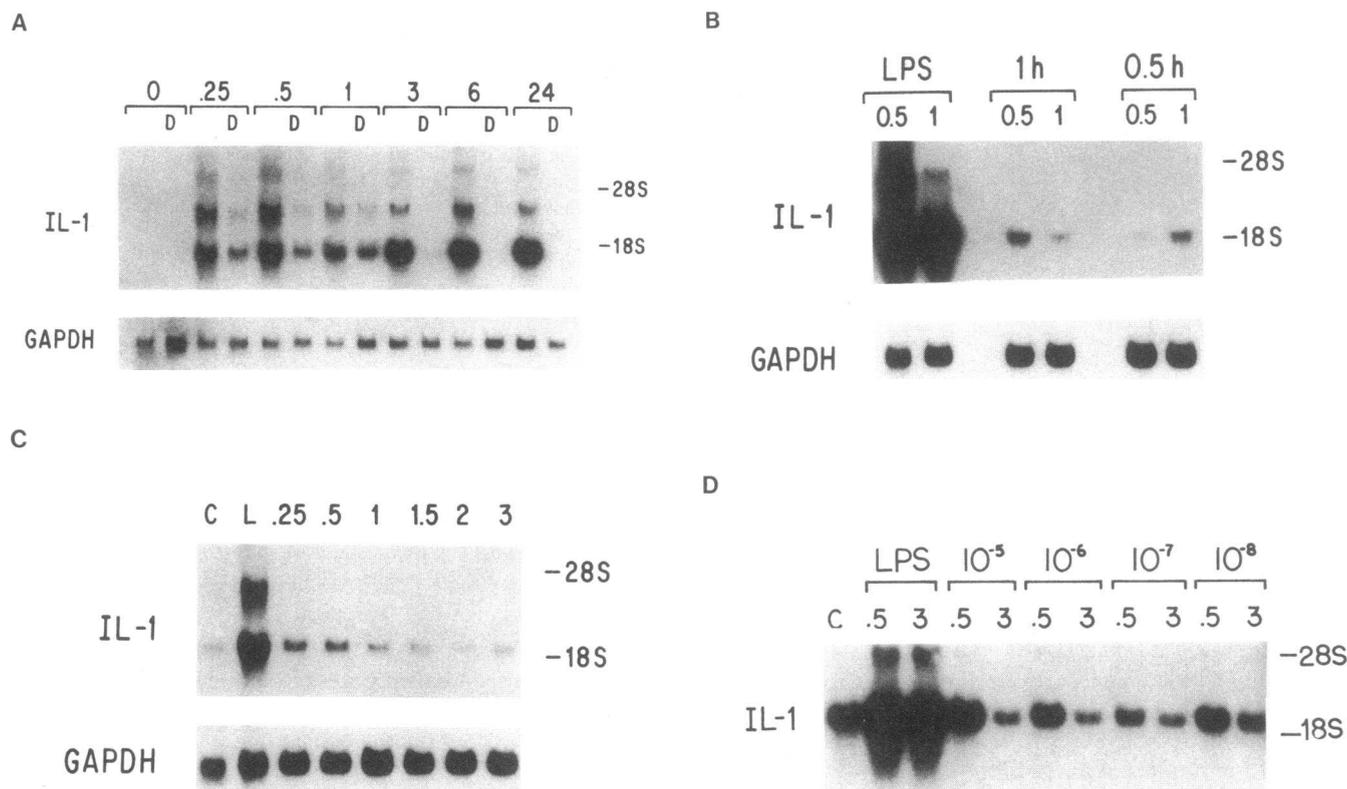
The effects of dexamethasone on LPS induction of another cytokine in U-373 cells, IL-6, was also studied at 37°C and 40°C. Dexamethasone suppressed this induction maximally at a concentration of  $10^{-5}$  M and was more effective at 37°C than at 40°C (Fig. 9 B).

**Effects of indomethacin on LPS-induced IL-1 $\beta$  and TNF $\alpha$  mRNA expression.** To determine if an antiinflammatory agent other than dexamethasone might similarly inhibit LPS-induced IL-1 $\beta$  and TNF $\alpha$  mRNA expression, U-373 cells were simultaneously treated at 37°C and 40°C with LPS and indomethacin, an inhibitor of prostaglandin biosynthesis. At 37°C, indomethacin had no effect on IL-1 $\beta$  mRNA expression (Fig. 10 A). The slightly increased signal in simultaneously treated cells corresponds to the total amount of RNA loaded on the gel. At 40°C, indomethacin slightly inhibited IL-1 $\beta$  mRNA induction, although not as extensively as dexamethasone (Fig. 10 A). Indomethacin did not inhibit LPS induction of TNF $\alpha$  mRNA expression at 37°C but was slightly inhibitory at 40°C (data not shown). Simultaneous exposure of LPS treated U-373 cells to dexamethasone and indomethacin was no more effective in suppressing IL-1 $\beta$  or TNF $\alpha$  mRNA expression than using either agent alone (data not shown). U-87 cells were similarly



**Figure 6.** Effects of dexamethasone treatment on LPS induction of IL-1 $\beta$  protein synthesis in U-373 cells. U-373 cells cultured at 37°C were treated with LPS 500 ng/ml (circles) or LPS plus dexamethasone  $10^{-5}$  M (squares) and cell extracts were

harvested at the indicated time points. IL-1 $\beta$  protein (in picograms per  $10^6$  cells) was measured by ELISA as described in Methods.  $1 \times 10^6$  cells were used for each experiment.



**Figure 7.** Effects of dexamethasone treatment on LPS induction of IL-1 $\beta$  mRNA expression in U-87 cells. (A) U-87 cells were treated with LPS or LPS plus dexamethasone (indicated by D) and cultured at 37°C. Numbers refer to the time in hours when cells were harvested. 20  $\mu$ g of total cellular RNA from cells at each time point was separated by gel electrophoresis, transferred to a nylon membrane, and hybridized first to a <sup>32</sup>P-labelled human IL-1 $\beta$  cDNA probe and subsequently to a GAPDH probe. The large molecular weight bands are unprocessed and partially processed IL-1 $\beta$  nuclear RNA precursors. (B) U-87 cells were pretreated with dexamethasone for 0.5 or 1 h (numbers above brackets) and then exposed to LPS. LPS above brackets indicates cells treated with LPS alone and not pretreated with dexamethasone. IL-1 $\beta$  mRNA expression was assayed by Northern blot analysis of RNA from cells harvested at 0.5 and 1 h after exposure to LPS (numbers below brackets). The location of the 28S and 18S rRNA bands are indicated. GAPDH served as an internal concentration control. (C) U-87 cells were treated with LPS for 0.5 h before the administration of dexamethasone. IL-1 $\beta$  mRNA expression was assayed by Northern blot analysis of RNA from cells harvested at the times indicated. C, control for endogenous expression of IL-1 $\beta$ ; L, cells after 0.5 h treatment with LPS alone. The numbers correspond to the time in hours when cells were harvested. The location of the 28S and 18S rRNA bands are indicated. GAPDH served as an internal RNA concentration control. (D) U-87 cells were treated with LPS alone or LPS plus dexamethasone and harvested after 0.5 and 3 h. The numbers above the brackets correspond to the concentration of dexamethasone in moles per liter.

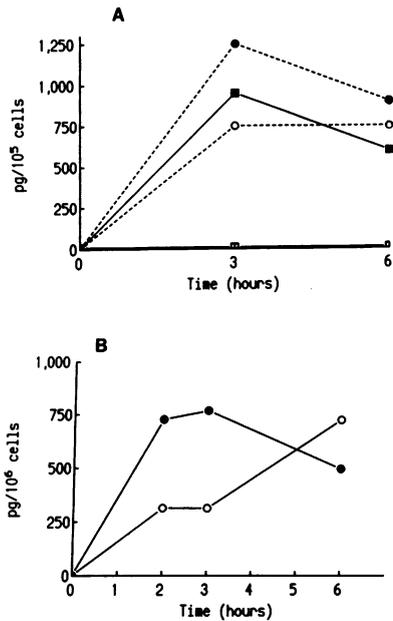
simultaneously treated with LPS and indomethacin. Indomethacin extensively inhibited IL-1 $\beta$  mRNA at 0.5 and 3 h (Fig. 10 B).

## Discussion

Results from animal studies and clinical trials have clearly shown that the cytokines IL-1 $\beta$  and TNF $\alpha$  act synergistically as initiators of inflammation in bacterial meningitis (6, 10). Our findings demonstrate that astrocytoma cell lines and primary cultures of fetal astrocytes are useful systems in which to investigate molecular aspects of expression of these cytokines. While comparisons between cultured transformed human tumor cell lines and the normal cells from which the tumor is derived (in this case astrocytes) may be limited, our studies with primary cell cultures support our observations in astrocytoma cell lines which suggest that astrocytes are at least one source of IL-1 $\beta$  and TNF $\alpha$  that has been detected in the CSF from patients with bacterial meningitis (10). The kinetics of IL-1 $\beta$  mRNA induc-

tion varied in the two astrocytoma cell lines and primary astrocyte cultures depicted, as well as in seven other astrocytoma cell lines that were tested (Velasco, S., and P. Nisen, unpublished observations). Furthermore, TNF $\alpha$  expression in LPS-treated astrocytoma cells could only be detected in the U-373 cell line and primary fetal astrocytes. These variations may be a result of differences in the relative state of differentiation of the cells or the type of astrocyte from which the tumor cell lines arose. Overall, our findings are consistent with observations in an animal model of meningitis in which IL-1 $\beta$  and TNF $\alpha$  were initially detectable in the CSF within 2 h of exposure to LPS (7, 8).

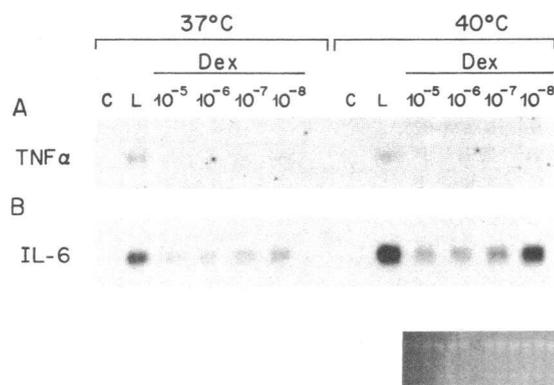
We found that increased temperature blunted LPS induction of IL-1 $\beta$  and TNF $\alpha$  in astroglial cells. The induction of multiple other cytokines and growth factors synthesized by astroglial cells in response to LPS at 37°C was diminished when cells were cultured at 40°C (Velasco, S., and P. Nisen, unpublished observations). It has recently been reported that inducers of the heat shock response (i.e., elevated temperature) downre-



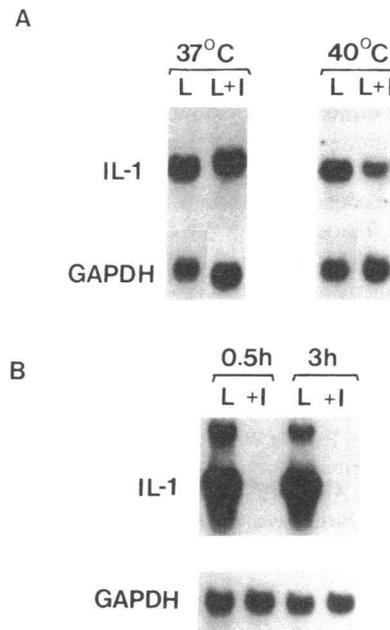
**Figure 8.** Effects of LPS and dexamethasone on IL-1 $\beta$  and TNF $\alpha$  protein synthesis in primary cultures of human fetal astrocytes. Primary human fetal astrocytes (A) and SV40 T antigen transfected human fetal astrocytes (B) were treated with LPS 500 ng/ml (closed circles and squares) or LPS 500 ng/ml plus dexamethasone  $10^{-5}$  M (open circles and squares). IL-1 $\beta$  protein (circles) and TNF $\alpha$  protein (squares) were measured by ELISA as outlined in Methods.  $2 \times 10^4$  cells were used for each experiment in A, and  $1 \times 10^6$  cells were used for

experiment in B. TNF $\alpha$  was not detectable in LPS-treated SV40 T antigen transfected human fetal astrocytes.

regulated IL-1 $\beta$  biosynthesis in a human myelomonocytic cell line (19). It is conceivable that humans use a feedback inhibition mechanism in which the induction of IL-1 $\beta$  and TNF $\alpha$  causes fever that activates heat shock proteins which, in turn, downregulate IL-1 $\beta$  and TNF $\alpha$ . Such a feedback inhibition loop could potentially balance the beneficial protective effects of these cytokines and the deleterious sequelae of an exaggerated host inflammatory response.



**Figure 9.** Dexamethasone suppression of TNF $\alpha$  and IL-6 mRNA induction by LPS at 37°C and 40°C. RNA was extracted from U-373 cells after 3 h treatment with LPS 500 ng/ml (L) or LPS plus dexamethasone (Dex). Dexamethasone concentrations are listed in moles per liter. Cells were incubated at 37°C or 40°C as indicated by the brackets. C indicates control cells not treated with LPS. (A) Northern blot hybridization analysis using a TNF $\alpha$  cDNA probe; (B) Northern blot hybridization analysis (after stripping) using a human IL-6 cDNA probe. A photograph of the ultraviolet light-illuminated, ethidium bromide-stained gel depicting the signal intensity of the 28S and 18S rRNA bands demonstrates that equal amounts of RNA were loaded in each lane.



**Figure 10.** Effects of indomethacin on IL-1 $\beta$  mRNA induction by LPS. (A) U-373 cells were treated with LPS alone (L) or LPS plus indomethacin (L + I). RNA from cells harvested after 3 h at 37°C or 40°C was assayed for IL-1 $\beta$  mRNA expression by Northern blot analysis. GAPDH served as an internal RNA concentration control. (B) U-87 cells were treated with LPS alone (L) or LPS plus indomethacin (L + I). RNA from cells harvested after 0.5 or 3 h was assayed for IL-1 $\beta$  mRNA expression by Northern blot analysis. GAPDH served as an internal RNA concentration control.

The results of animal studies and clinical trials demonstrated that dexamethasone has a protective effect in bacterial meningitis that may result, at least in part, from suppression of IL-1 $\beta$  and TNF $\alpha$  in the CNS (8, 11). Our findings reveal a potential mechanism by which this occurs. Dexamethasone suppresses LPS induction of IL-1 $\beta$  and TNF $\alpha$  mRNA transcription in astroglial cells. Similar inhibitory effects of dexamethasone on IL-1 $\beta$  and TNF $\alpha$  mRNA expression have been observed in monocytes/macrophages (2, 20).

The results of animal and clinical studies also indicated that the timing of administration of dexamethasone in relation to initiating antibiotic therapy was important in inhibiting cytokine induction and consequent meningeal inflammation as well as in improving outcome from disease (5, 21). Data from clinical trials in patients with bacterial meningitis indicate that while it is preferable to administer dexamethasone before the first dose of antibiotic therapy (and the consequent release of LPS from lysed bacterial cells) (13), steroid therapy still has a beneficial effect if given subsequent to the first antibiotic dose (12). Our findings corroborate these data. We also found that suppression of LPS induction of IL-1 $\beta$  is dependent on the dosage of dexamethasone, an observation that is also consistent with the findings from clinical trials and animal studies that suggested that the effects of dexamethasone were dose dependent.

Dexamethasone was somewhat more effective in suppressing LPS induction of IL-1 $\beta$  mRNA at 37°C than at 40°C. Interaction of heat shock proteins with the glucocorticoid receptor are thought to downregulate responsiveness to glucocorticoids such as dexamethasone (19). This may explain why dexamethasone suppression was less effective at the higher temperature.

The effects of indomethacin on LPS induction of IL-1 $\beta$  are more complex. While IL-1 $\beta$  induction by LPS was clearly inhibited

ited by indomethacin in one cell line, a second cell line demonstrated temperature dependence. At normal body temperatures (37°C), indomethacin was ineffective, whereas at a higher temperature (40°C) it was inhibitory. This finding may be explained by the activation of different signal transduction pathways at different temperatures.

In conclusion, we used human astrocytoma tumor cell lines and primary cultures of human fetal astrocytes to characterize LPS induction of IL-1 $\beta$  and TNF $\alpha$  in cell culture at two temperatures, and determined the effects of dexamethasone and indomethacin on this process. These findings provide an explanation, at the molecular level, why dexamethasone (and perhaps other antiinflammatory agents) and possibly fever are beneficial in bacterial meningitis.

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