Interleukin-1β Expression after Inhibition of Protein Phosphatases in Endotoxin-Tolerant Cells

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Received 1 October 1997/Returned for modification 18 December 1997/Accepted 21 January 1998

Endotoxin (lipopolysaccharide [LPS]) is a potent activator of a number of inflammatory genes in blood leukocytes, including interleukin-1 (IL-1). Blood leukocytes isolated from patients with septic shock fail to produce IL-1 in response to LPS, a phenomenon known as endotoxin tolerance. To study the regulation of IL-1 expression in endotoxin-tolerant cells, the protein phosphatase inhibitor okadaic acid was used to examine the effects of protein phosphorylation on IL-1β gene expression. We found that endotoxin-tolerant cells produced normal levels of IL-1β when protein phosphatases were inhibited. In the human pro-monocytic cell line THP-1, okadaic acid increased mRNA accumulation and synthesis of IL-1β protein. Normal and endotoxin-tolerant THP-1 cells accumulated IL-1β mRNA and protein with similar delayed kinetics. Okadaic acid stabilization of IL-1β mRNA appears to be the primary mechanism through which endotoxin-tolerant cells accumulate IL-1β mRNA and protein. Endotoxin-tolerant cells were unable to activate transcription in response to okadaic acid. However, the transcription factor NF-κB, which is known to be involved in IL-1β expression, was translocated to the nucleus in both normal and endotoxin-tolerant cells after treatment with okadaic acid. These studies revealed that protein phosphorylation can affect gene expression on at least two distinct levels, transcription factor activation and mRNA stability. Endotoxin-tolerant cells have decreased transcription activation potential, while IL-1β mRNA stability remains responsive to protein phosphorylation.

Septic shock is a lethal syndrome and the principal cause of death in patients in intensive care units (26). A number of microbial products, including bacterial lipopolysaccharide (LPS), cause septic shock by inducing the expression of proinflammatory genes. Phagocytic cells, monocytes and neutrophils, respond to LPS by producing the potent immune and inflammatory mediator interleukin-1 (IL-1). Blood leukocytes isolated from patients with septic shock fail to produce IL-1 in response to LPS, a phenomenon known as endotoxin tolerance. To study the regulation of IL-1 expression in endotoxin-tolerant cells, the protein phosphatase inhibitor okadaic acid was used to examine the effects of protein phosphorylation on IL-1β gene expression. We found that endotoxin-tolerant cells produced normal levels of IL-1β when protein phosphatases were inhibited. In the human pro-monocytic cell line THP-1, okadaic acid increased mRNA accumulation and synthesis of IL-1β protein. Normal and endotoxin-tolerant THP-1 cells accumulated IL-1β mRNA and protein with similar delayed kinetics. Okadaic acid stabilization of IL-1β mRNA appears to be the primary mechanism through which endotoxin-tolerant cells accumulate IL-1β mRNA and protein. Endotoxin-tolerant cells were unable to activate transcription in response to okadaic acid. However, the transcription factor NF-κB, which is known to be involved in IL-1β expression, was translocated to the nucleus in both normal and endotoxin-tolerant cells after treatment with okadaic acid. These studies revealed that protein phosphorylation can affect gene expression on at least two distinct levels, transcription factor activation and mRNA stability. Endotoxin-tolerant cells have decreased transcription activation potential, while IL-1β mRNA stability remains responsive to protein phosphorylation.

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MATERIALS AND METHODS

Cell culture and induction of endotoxin tolerance. THP-1 cells (a human pro-monocytic cell line) (American Type Culture Collection, Rockville, Md.) were cultured in RPMI 1640 medium (Gibco BRL, Gaithersburg, Md.) supplemented with 10 U of penicillin G per ml, 10 μg of streptomycin per ml, 2 mM L-glutamine, and 10% fetal calf serum (HyClone Laboratories, Logan, Utah). THP-1 5A cells, a generous gift from John G. Gray (Department of Molecular Genetics, Glaxo Wellcome, Inc., Research Triangle Park, N.C.) are THP-1 cells stably transfected with pIL-1 (4.0 kb)–secreted placental alkaline phosphatase (SPAP) (19). Transient transfections with a plasmid containing six NF-κB binding sites and a chloramphenicol acetyltransferase (CAT) reporter gene were performed as previously described (42). Endotoxin (LPS) tolerance was induced for both THP-1 cells and THP-1 5A cells as previously described (17). Briefly, cells were made tolerant with a primary dose of LPS (1 μg of LPS [E. coli O111: B4; Sigma Chemical Co., St. Louis, Mo.] per ml) for 18 h. The cells were then pelleted, washed once, and stimulated as described in legends to the figures. For all experiments, normal cells were treated similarly but were not given the primary LPS dose. A complete characterization of the tolerant THP-1 model has been previously published (17).

RNA isolation and Northern blot analysis. Total RNA was isolated from cells by using RNA STAT-60 (Tel-Test “B”, Inc., Friendswood, Tex.) according to the
manufactur er's instructions. After transfer to nylon membranes, IL-1β and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were visualized by autoradiography as described previously (17).

**IL-1β enzyme-linked immunosorbent assay (ELISA).** Total IL-1β protein (intracellular and secreted) was assessed with an IL-1β immunoassay kit (sensitivity range, 5 to 1,000 pg of IL-1/β/ml; Immunotchn, Inc., Westbrook, Maine) according to the manufacturer's instructions. Samples were obtained by lysis of cells plus media in RIPA buffer (150 mM NaCl, 1% deoxycholate, 1% Triton, 0.1% sodium dodecyl sulfate, 10 mM Tris [pH 7.4]), cleared by centrifugation, assayed in duplicate, and read on a Thermomax microplate reader (Molecular Devices, Sunnyvale, Calif.). Data were analyzed and plotted with Microsoft Excel (version 5.0).

**IL-1β mRNA half-life determination.** Normal and tolerant THP-1 cells (10^6 ml) were stimulated with LPS (1 μg/ml) or okadaic acid (100 nM) for 3 or 6 h, respectively. Following stimulation, the cells were treated with actinomycin D (5 μg/ml) (Sigma Chemical Co.), and total RNA was prepared at various times after addition of actinomycin D. IL-1β mRNA half-life was assessed by Northern blot and quantitated with a PhosphorImager 425 SI (Molecular Dynamics) and ImageQuaNT 4.1 software (Molecular Dynamics). IL-1β mRNA levels were normalized to GAPDH mRNA levels and expressed as percent mRNA by using Microsoft Excel software (version 5.0). Alternatively, RNA half-life was determined by reverse transcription (RT) of sample RNA and amplification of the reverse-transcribed cDNA with the GeneAmp PCR kit (Perkin-Elmer, Foster City, Calif.) and the GeneAmp PCR System 9600 (Perkin-Elmer Cetus, Emeryville, Calif.) according to the manufacturer's instructions. The primers used were IL-1β 5′ primer, 5′-GCAAGGGCTTCAGGCGGCGG-3′; IL-1β 3′ primer, 5′-GTCATCCCTGAGGCTGTGAC-3′; GAPDH 5′ primer, 5′-CATGGAAGGCTGCTGGG-3′; and GAPDH 3′ primer, 5′-CAA AGTGTCTATGATGAC-3′. Times and temperatures for the RT cycle were 60 min at 42°C, 5 min at 98°C, and 5 min at 4°C. The PCR was performed in the presence of 5 μCi of [γ-32P]ATP, and the reverse-transcribed cDNA was amplified for 1 min at 97°C, 1 min at 55°C, and 1 min at 72°C for each of 15 cycles and a final hold for 5 min at 72°C. Radiolabeled PCR products were resolved on an 8% polyacrylamide gel, quantitated with a Molecular Dynamics PhosphorImager 425 SI and ImageQuaNT 4.1 software, normalized to GAPDH mRNA levels, and expressed as percent mRNA by using Microsoft Excel software (version 5.0).

**SPAP assays.** SPAP was assayed from THP-1 5A cell culture supernatants with the Great Escape detection kit (Clontech, Palo Alto, Calif.) according to the manufacturer’s instructions. Data were analyzed and plotted with Microsoft Excel software (version 5.0).

**Nuclear extract preparation and EMSA.** Following treatment as described in the figure legends, THP-1 cells were harvested and nuclear extracts were prepared and stored at −70°C for use in electrophoretic mobility shift assays (EMSAs) as previously described (42). Oligonucleotide probes for the binding sites of NF-kB and octamer 1 were synthesized by using an Applied Biosystems model 380B automated DNA synthesizer as previously described (42). Specificity of DNA binding was also determined as previously described (42; also, data not shown).

**RESULTS**

**IL-1β mRNA expression in endotoxin-tolerant cells as a result of phosphatase inhibition.** Phosphatase inhibition by okadaic acid has been reported to induce transcription of IL-1β in human monocytes (36). To determine whether okadaic acid could induce IL-1β expression in our endotoxin-tolerant-cell model, normal and tolerant cells were stimulated for various times with 100 nM okadaic acid and IL-1β mRNA was visualized on a Northern blot of total RNA (Fig. 1A). As we had observed previously (17), endotoxin-tolerant cells were impaired in their ability to induce IL-1β mRNA expression when challenged with LPS (Fig 1; compare lanes L). However, okadaic acid induced IL-1β mRNA in both normal and tolerant cells with maximal accumulation at approximately 6 h in both phenotypes. These results show that okadaic acid can induce IL-1β mRNA in tolerant cells, where IL-1β expression in response to LPS is impaired.

**Okadaic acid increases IL-1β expression in endotoxin-tolerant cells.** As shown in Fig. 1B, okadaic acid induced similar amounts of total IL-1β protein in normal and tolerant cells. The kinetics of IL-1β protein accumulation correlates with the accumulation of IL-1β mRNA, and tolerant cells do not show the lag phase observed in normal cells in their response to okadaic acid.

**Okadaic acid increases IL-1β mRNA half-life.** While LPS-induced IL-1β mRNA expression is rapid and transient (10, 17), LPS-stimulated IL-1β mRNA in THP-1 cells has half-lives of 50 and 100 min in normal and tolerant cells, respectively (Fig. 2). In contrast, okadaic acid treatment resulted in a delayed and prolonged expression of IL-1β transcripts (Fig. 1A) in both normal and tolerant phenotypes. Okadaic acid could increase IL-1β mRNA levels through increased transcription or stabilization of IL-1β mRNA, mechanisms both of which are known to be important in the regulation of IL-1β expression (1). To determine the effect of phosphatase inhibition on IL-1β mRNA stabilization, normal and tolerant cells were treated with 100 nM okadaic acid for 6 h, at which time further transcription was inhibited by the addition of 5 μg of actinomycin D per ml. Total RNA was isolated at various times after addition of actinomycin D, and IL-1β mRNA was quantitated by RT-PCR (Fig. 3A) or on Northern blots (Fig. 3C) with similar results (Fig. 3B and D, respectively). Okadaic acid stabilized IL-1β mRNA in normal and tolerant cells (half-life, >2 h), with no degradation of IL-1β mRNA observed over the times tested.

**Phosphatase inhibition by okadaic acid does not activate transcription of reporter genes in tolerant cells.** To determine the role of transcription activation in okadaic acid-induced IL-1β expression, we used an SPAP reporter gene linked to enhancer/promoter elements from the IL-1β gene. Normal and endotoxin-tolerant cells stably transfected with pIL-1 (4.0 kb)–SPAP, THP-1 5A cells (19), were stimulated with 100 nM
okadaic acid, and transcription activation was measured by expression of SPAP in the cell culture supernatant. Figure 4A shows that LPS strongly induced transcription of the SPAP reporter gene in normal cells and that this induction was repressed in a tolerant cell. This result confirms and extends our previous observation of repressed transcription in endotoxin-tolerant cells (17). We also found that, although okadaic acid is a less potent inducer of SPAP transcription relative to LPS, okadaic acid-induced SPAP expression was similarly repressed in a tolerant cell. Control experiments showed that okadaic acid had no direct effect on the phosphatase assays used to measure SPAP reporter gene expression (data not shown). As expected, LPS did not induce IL-1β protein in tolerant cells compared to the normal cells, while okadaic acid induced similar amounts of IL-1β protein in both phenotypes (Fig. 4B). These results show that endotoxin-tolerant cells are repressed at the level of transcription in that both LPS and okadaic acid are unable to induce SPAP reporter gene expression through promoter/enhancer sequences of the IL-1β gene. These results suggest that, in endotoxin-tolerant cells, IL-1β mRNA accumulation in response to phosphatase inhibition by okadaic acid occurs through a mechanism which is independent of transcription activation. LPS stimulation and phosphatase inhibition are apparently unable to activate transcription of the IL-1β gene in endotoxin-tolerant cells.

Okadaic acid does not activate NF-κB-dependent transcription in endotoxin-tolerant cells. The expression of IL-1β has been shown to be dependent on the activation of the transcription factor NF-κB (6, 14). NF-κB is a potential regulatory target in the activation/repression we have observed in the normal and tolerant phenotypes. We therefore sought to determine the effect of phosphatase inhibition on NF-κB-dependent transcription in normal and endotoxin-tolerant cells. THP-1 cells were transiently transfected with a CAT reporter gene containing an enhancer of six NF-κB binding sites (42). Normal- and endotoxin-tolerant-cell lysates were assayed for CAT activity at various times after addition of okadaic acid. Figure 5A shows okadaic acid induction of NF-κB-dependent transcrip-
Okadaic acid activates NF-κB nuclear translocation in normal and endotoxin-tolerant cells. Okadaic acid had previously been shown to induce NF-κB nuclear translocation and activation (29, 39). Given the apparent inability of okadaic acid to induce NF-κB-dependent transcription activity in endotoxin-tolerant cells, we determined whether okadaic acid could mediate the nuclear localization of NF-κB in the tolerant phenotype. Nuclear extracts were prepared from okadaic acid-treated normal and tolerant cells over a time course of 9 h, and NF-κB binding activity was determined by EMSA. As shown in Fig. 5B, the nuclear translocation and DNA binding activity of NF-κB in nuclear extracts from control and tolerant cells were increased in response to okadaic acid. In normal cells, nuclear translocation appears to correlate with the induction of NF-κB-dependent CAT reporter gene transcription (Fig. 5A). Tolerant cells have an increased basal level of NF-κB binding (Fig. 5B; compare lanes 0) due to some induction by LPS during the tolerizing step; increased nuclear localization and DNA binding of NF-κB by okadaic acid do not, however, result in high levels of CAT activity.

These results show that phosphatase inhibition can induce nuclear localization of NF-κB in normal and tolerant cells. Despite the quantitatively similar nuclear localization in normal and tolerant cells, NF-κB-dependent transcription activation is not observed in the tolerant phenotype. However, there may be qualitative differences (43) in DNA binding in the tolerant phenotype (Fig. 5B; compare the relative increase in the amount of the upper NF-κB-specific protein-DNA complex in normal cells with the relative increases in the amounts of both upper and lower NF-κB-specific protein-DNA complexes in tolerant cells). The nature of this qualitative difference was not further investigated. Taken together, these results indicate that phosphatase inhibition stabilizes IL-1β mRNA and is the predominant mechanism by which IL-1β can be induced in normal and endotoxin-tolerant cells. THP-1 cells and/or THP-1 5A cells (10^6/ml) were stimulated with OA (100 nM) for 6, 10, or 18 h, and total IL-1β protein was measured by ELISA as described in Materials and Methods. The amount of IL-1β protein was used as 100% and compared to tolerant-cell IL-1β protein levels. Error bars indicate standard errors of the means (SEM) for three experiments. Average actual values for 100% are indicated below. (B) Normal and endotoxin-tolerant cells were stimulated with LPS (100 ng/ml) for 18 h to induce tolerance. Normal and endotoxin-tolerant cells were also treated with LPS (100 ng/ml) to determine relative IL-1β protein levels. Error bars indicate SEM of 11 experiments. Normal and endotoxin-tolerant cells were also treated with LPS (1 μg/ml) to verify the tolerant phenotype at each time tested.

Okadaic acid activates NF-κB nuclear translocation in normal and endotoxin-tolerant cells. Okadaic acid had previously been shown to induce NF-κB nuclear translocation and activation (29, 39). Given the apparent inability of okadaic acid to induce NF-κB-dependent transcription activity in endotoxin-tolerant cells, we determined whether okadaic acid could mediate the nuclear localization of NF-κB in the tolerant phenotype. THP-1 cells and/or THP-1 5A cells (10^6/ml) were treated with LPS (1 μg/ml) for 18 h to induce tolerance. (A) Relative AP activity from normal and endotoxin-tolerant THP-1 5A cells (THP-1 cells stably transfected with pIL-1 [4.0 kb]-SPAP). At 6 or 18 h after okadaic acid (OA) stimulation, cell supernatants were assayed for AP activity as described in Materials and Methods. For each time, normal-cell AP activity was used as 100% and compared to tolerant-cell AP activity to determine relative AP activity.

Materials and Methods. For each time, normal-cell AP activity was used as 100% and compared to tolerant-cell AP activity to determine relative AP activity. Normal and endotoxin-tolerant cells were also treated with OA (100 nM) for 6, 10, or 18 h, and total IL-1β protein was measured by ELISA as described in Materials and Methods. For each time, normal-cell AP activity was used as 100% and compared to tolerant-cell AP activity to determine relative AP activity.
expressed in a tolerant cell where a repressor (17) may inhibit high levels of increased transcription activation.

**DISCUSSION**

LPS induction of IL-1β is rapid and transient, and the kinetics of IL-1β production can be physiologically related to the inflammatory response induced by this potent bacterial toxin. Okadaic acid, in contrast, is a potent tumor promoter whose growth-regulatory mechanisms do not require such immediate physiological responses. Early studies showed that okadaic acid mimicked protein phosphorylation and gene expression patterns induced by IL-1 and TNF-α, two lymphokines with definitive growth-regulatory properties—IL-1 as a comitogen for thymocyte maturation and TNF-α as a cytotoxic agent for tumor cells (12). We (this study) and others (36, 38) have shown that okadaic acid increased IL-1β production in monocytes. We further show that, in a normal cell, protein phosphorylation can affect IL-1β expression on at least two levels, transcription activation and mRNA stability. Previous studies by Sung and Walters (36) also showed increases in transcription of the IL-1β gene; however, they did not observe any effect of okadaic acid on IL-1β mRNA stability compared to LPS-induced IL-1β mRNA. In contrast, we find a significant change in mRNA stability in the presence of okadaic acid (half-life, >2 h) compared to mRNA stability in the presence of LPS (half-life, <2 h). The discrepancy between our results and those of Sung and Walters is most likely due to the time at which mRNA stabilization was assessed; Sung and Walters measured IL-1β mRNA stability at only 2 h of okadaic acid treatment, a time which, we have shown, is less than optimal for inhibition of phosphatase activity (data not shown) and peak induction of IL-1β mRNA accumulation by okadaic acid (Fig. 1).

We confirm and extend our previous studies showing that IL-1β expression in endotoxin-tolerant cells is repressed at the level of transcription (17), while mRNA stability remains responsive to regulation by protein phosphorylation. Our results indicate that these two regulatory mechanisms in IL-1β expression, transcription activation and mRNA stability, are distinct and separable. Transcription repression appears to be the dominant defect in endotoxin-tolerant cells. Okadaic acid, like LPS, is unable to activate transcription of the IL-1β gene in endotoxin-tolerant cells. We have observed, however, that some inducers of IL-1β, cycloheximide for example, are able to overcome transcription repression, perhaps by inhibiting the synthesis of a transcription repressor (17). Despite the inability to induce high levels of IL-1β transcription, endotoxin-tolerant cells can produce normal levels of IL-1 when protein phosphatases are inhibited. IL-1β mRNA, which is transcribed at basal levels in normal untreated and in endotoxin-tolerant cells, becomes stabilized by okadaic acid, resulting in a slow accumulation of IL-1β mRNA and synthesis of similar levels of IL-1β protein (Fig. 1).

The IL-1β gene is expressed in response to a number of stimulants in addition to LPS and okadaic acid, including phorbol myristate acetate, cyclic AMP, and cytokines, such as TNF-α, IL-6, and IL-1 itself (reviewed in reference 1). Accordingly, a number of signal transduction pathways regulate the expression of IL-1. For example, LPS induces activation of p38, a member of the mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK) family of protein kinases (13). p38 MAP kinases, in turn, regulate intracellular events including activation of transcription factors such as ATF-2 (27) and Elk-1 (28) and mRNA translation (18). The requirement for dual phosphorylation on adjacent tyrosine and threonine residues for activation is a salient feature of MAPK/ERK family members. Okadaic acid activates MAPK/ERK p42 (5, 22), but whether these kinases are directly or indirectly involved in the regulation of mRNA stabilization by okadaic acid is not known. A recent study using another serine/threonine phosphatase inhibitor, calyculin A, suggested that MAPK activation alone is not sufficient for induction of IL-1β (2).

While the role of protein kinases in signal transduction has been well recognized, protein phosphatases have only recently emerged as important regulators of cellular function (7, 37). Our understanding of phosphatases in cellular regulation has progressed from the assumption of a passive role for phosphatases in returning kinase-activated cascades to equilibrium to one of a dynamic signal transduction mechanism. This insight has been provided largely through studies employing serine- and threonine-specific protein phosphatase inhibitors, such as okadaic acid, and the identification, purification, and cloning of their specific phosphatase targets (30). More recently, a number of tyrosine (reviewed in reference 33) and dual-specificity (tyrosine and serine/threonine) phosphatases have also been identified (16, 21, 23). Collectively, the number, specificity, and cyclic nature of the phosphorylation-dependent regulatory mechanisms enables diversity in response to physiological stimuli. Our studies emphasize an important role for protein phosphatases in the regulation of mRNA stability, particularly in the absence of the ability to induce transcription. Endotoxin-tolerant cells, while unable to initiate new transcription of the IL-1β gene, can utilize basal levels of expression to produce normal levels of IL-1 through phosphorylation-dependent mechanisms. This regulatory pathway could be important as a survival mechanism in animal models of sepsis: the endotoxin-resistant mouse strain C3H/HeJ does not develop rapid inflammatory responses and has a significantly decreased ability to resist infections (8). Utilization of phosphatase inhibitors might increase survival in these animals by restoration of some measure of cytokine-mediated host defense. We have observed that peripheral blood mononuclear cells from a septic patient (endotoxin tolerant) and a healthy individual produce similar levels of IL-1β when treated ex vivo with okadaic acid (data not shown). Further studies on septic patients and animal models of endotoxin tolerance are necessary to evaluate the therapeutic value of phosphatase inhibition.

The mechanisms involved in tolerance to LPS are largely unknown but do not appear to involve down-regulation or alteration of LPS receptors on the cell surface (9). We did not observe any change in total phosphatase activity in tolerant cells (data not shown), and the inactivation of phosphatases by okadaic acid follows a similar time course in normal and tolerant cells. The similarly delayed kinetics of NF-κB induction and IL-1β mRNA and protein accumulation in response to okadaic acid further suggest that some signal transduction pathways (e.g., those sensitive to serine/threonine dephosphorylation by protein phosphatases 1 and 2A, two phosphatases directly inhibited by okadaic acid), are intact in endotoxin-tolerant cells. Recent evidence suggests that NF-κB activation by okadaic acid is indirect and mediated by the induction of reactive oxygen intermediates rather than through directly influencing the phosphorylation state of IκB (29). Whether direct or indirect, okadaic acid induction of IL-1β appears not to be altered in the endotoxin-tolerant phenotype. IL-1β mRNA half-life was somewhat prolonged in LPS-stimulated tolerant cells. This increase in mRNA stability did not result in an accumulation of mRNA (Fig. 1A; compare lanes L). This contrasts with the pronounced effect of okadaic acid on IL-1β mRNA stability, which leads to increases in the levels of IL-1β.
mRNA and IL-1β protein (Fig. 1B and 4B). Taken together, these observations emphasize the importance of a transcriptional defect in maintaining the endotoxin-tolerant phenotype (17).

Results of this study provide further insight into the nature of the transcriptional defect in endotoxin-tolerant cells. The basal level of NF-κB binding is increased in the tolerant phenotype, and okadaic acid mediates a further increase in NF-κB. However, transcription activation by NF-κB remains repressed despite an increase in binding of the putative transcription factors. Similarly, the stably transfected SPAP reporter linked to enhancer/promoter elements from the IL-1β gene (which contains several NF-κB enhancer elements, in addition to other transcription regulatory elements) was also repressed in the tolerant phenotype and did not respond to okadaic acid. A recent study of blood mononuclear cells obtained from patients with sepsis showed increases in NF-κB binding (3). Increased NF-κB binding correlated with increased mortality. We have also observed increased binding of NF-κB in polymorphonuclear cells from patients with severe sepsis (unpublished observations). Polymorphonuclear cells from septic patients do not induce IL-1β expression in response to endotoxin (20). Collectively, the data support a model of transcription inhibition which is independent of the binding of transcription factors to their regulatory elements. The tolerant phenotype may involve qualitative differences in transcription factors, as suggested by a study with Mono-Mac-6 cells (43) and/or may involve other components of the transcriptional apparatus (e.g., transcription coactivators).

The control of mRNA degradation is a major contributor to the regulation of expression of many cytokines and protooncogenes. A common feature of these and other rapidly degraded mRNAs is the presence of multiple copies of AU-rich sequences in the 3′-untranslated region of the mRNA (32). These AU-rich motifs are thought to act as destabilizing elements, perhaps through interaction with a number of RNA-binding proteins which have been recently described (25). IL-1β and other inflammatory mediators, such as TNF-α, contain AU-rich sequences (4), although the roles of these elements and their cognate binding proteins in regulating mRNA stability are not yet known. Interestingly, it appears that no significant quantitative changes in the binding activity of AU-rich elements occur in response to several stimuli which stabilize mRNA (25), suggesting that perhaps mRNA stability is regulated by additional events, such as changes in protein phosphorylation of the RNA-binding proteins. We are currently examining whether okadaic acid may affect AU-rich binding proteins and determining the role these proteins may have in stabilization of IL-1β mRNA in normal and endotoxin-tolerant cells.

In summary, (i) okadaic acid facilitates IL-1β gene expression; (ii) okadaic acid regulates IL-1β mRNA stability, even in the endotoxin-tolerant phenotype; (iii) IL-1β mRNA stabilization in the endotoxin-tolerant phenotype results in the accumulation of mRNA and synthesis of IL-1β protein; (iv) the tolerant phenotype is associated with a defect in transcription activation of IL-1β which does not appear to be alleviated by okadaic acid; and (v) okadaic acid induces DNA binding of NF-κB in normal and tolerant cells; however, tolerant cells are unable to activate NF-κB-dependent transcription. This suggests that the tolerant phenotype may involve defects in other mechanisms that regulate transcription.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants HL-29293 and AI-09169 and General Clinical Research Center grant RR07122 from the National Institutes of Health.

THP-1 5A cells were a gift from John Gray, and the NF-κB/CAT reporter construct was a gift from Ed O’Neill.

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