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Regulation of Monokine Gene Expression: Prostaglandin E_2 Suppresses Tumor Necrosis Factor but Not Interleukin-1α or β-mRNA and Cell-Associated Bioactivity

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Prostaglandin E_2 (PGE_2)-mediated suppression of macrophage interleukin-1α,β and tumor necrosis factor-α synthesis was examined at the cellular and molecular levels. Treatment of lipopolysaccharide (LPS)-stimulated adjuvant-elicited murine macrophages with 5×10^{-7}M PGE_2 caused a 70% reduction in cell-associated TNF but had no suppressive effect on cell-associated interleukin-1 (IL-1) activity. Consistent with this result, Northern blot and nuclear transcription analyses demonstrated suppression of TNF mRNA but PGE_2 had no effect on IL-1α and IL-1β mRNA accumulation, as compared to LPS controls. Immunoperoxidase staining for cell-associated TNFα, IL-1α, and IL-1β demonstrated that PGE_2 suppressed TNF, but not IL-1α or -β expression, supporting the bioassay data. These results imply that PGE_2-mediated regulation of IL-1α,β and TNFα is quite distinct. Synthesis of TNF appears to be regulated at least at the level of transcription, whereas that for IL-1α and -β is regulated post-transcriptionally.

Key words: macrophage, inflammation, mediators

INTRODUCTION

Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are polypeptide products of macrophages that mediate a wide array of biological activities, involving a variety of target cell populations. Although these cytokines are biochemically and immunologically distinct, they share a number of common physiological functions, including the induction of fever, activation of neutrophils, inhibition of lipoprotein lipase, induction of procoagulant activity on endothelial cells, induction of surface antigens on endothelial cells, and activation of fibroblasts [1–5].

Macrophages are the major sources of IL-1α, IL-1β, and TNFα [6,7]. Interestingly, stimulation of macrophages with a polyclonal stimulant such as lipopolysaccharide (LPS) will result in the liberation of not only the above cytokines, but also products of arachidonic acid metabolism, including prostaglandin E_2 (PGE_2). Our laboratory and others have examined the potential role of PGE_2 in the modulation of macrophage cytokine production. We and others have demonstrated that PGE_2 causes a dose-dependent suppression of IL-1 and TNF release from LPS-stimulated macrophages [8–11]. These studies implicated a regulatory function of endogenously produced PGE_2 in the release of IL-1 and TNF from stimulated macrophages. In addition, the evidence that IL-1 or TNF alone will stimulate the release of PGE_2 from monocytes and macrophages [9,11,12] is consistent with an autoregulatory loop in which release of these monokines from stimulated macrophages will serve to induce an endogenous inhibitor of further cytokine release. The focus of the present study was to elucidate the mechanisms of PGE_2-mediated suppression of IL-1α, IL-1β, and TNFα release from stimulated murine macrophages by examining its effect on mRNA synthesis and the cellular accumulation of these activities.

MATERIALS AND METHODS

Animals and Macrophage Cultures

Adult female CBA/J H-2^k mice (Jackson Labs, Bar Harbor, ME) were maintained under pathogen-free conditions and given food and water ad libitum. Macrophages were elicited by the intraperitoneal injection of 0.5 ml of complete Freund's adjuvant (CFA) (Sigma

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Chemical Co., St. Louis, MO) mixed 1:1 with sterile saline. Peritoneal cells were harvested 2 wk later by aseptic peritoneal lavage with RPMI 1640 containing antibiotics (Gibco, Detroit, MI). Macrophages were allowed to adhere in 35-mm culture dishes at 37°C, 5% CO_2, 95% air for 2 h. Nonadherent cells were then removed by washing with warm RPMI, and the resulting monolayers were overlayed with media containing 1 μg/ml LPS (*Escherichia coli*, 0111:B4, Sigma Chemical Co., St. Louis, MO) or media alone. Previous studies in our laboratory have demonstrated adherent cells prepared in this way to be greater than 95% macrophages and greater than 80% Ia-positive. In prostaglandin experiments, monolayers were treated with prostaglandin E_2 (PGE_2) (kindly provided by Upjohn Co., Kalamazoo, MI), concomitant with LPS or control media. For monokine bioactivity determinations, the adherent cells were washed thrice with warm media, scraped into 1 ml of medium, and repeatedly frozen and thawed for assessment of cell-associated IL-1 and/or TNF.

Messenger RNA Analysis

At designated intervals after stimulation, total RNA was isolated from macrophage monolayers using a modification of the method of Chirgwin and Jonas [13,14]. RNA samples (10 μg/sample) were examined by Northern blot analysis, using formaldehyde 1% agarose gels. The separated RNA samples were transblotted to nitrocellulose, baked, prehybridized and hybridized using ^{32}P-labeled probes. The following oligonucleotides and cDNAs were utilized in this study: a) murine TNFα (30-mer) 5′-GTCCCCCTTCTCCAGCTGGAAGACTCCTCC-3′ [15]; b) murine IL-1α (26-mer) 5′-GTGAAGGTCTCACTGAAACTCAGCCG-3′ [16]; c) murine IL-1β (30-mer) 5′-TTCTATCTTGTTGAAGACAAACCGCTTTTC-3′ [17]; d) murine actin (42-mer) 5′-GGCTGGGGTGTTGAAGGTCTCAAACATGATCTGGGTCATCTT-3′ [18]; e) full-length cDNA probe for TNFα, (kindly provided by Cetus Corp., Emeryville, CA), [19]; and f) cDNA for murine IL-1α, (kindly provided by Dr. Peter Lomedico, Hoffman LaRoche, Nutley, NJ). Hybridized blots were washed and autoradiographed with intensifying screens.

Monokine Assays

Interleukin-1 levels were measured using the standard thymocyte coproliferation assay as modified from the procedure of Mizel et al. [20]. One unit of interleukin-1 activity was defined as the amount of material causing half-maximal stimulation in the co-proliferation assay.

The LM fibroblast cell line was used to measure levels of TNF according to a modification of the procedure of Ruff and Gifford [21]. Serial dilutions of test samples were added to 96-well microtiter plates (Costar, Cambridge, MA) containing LM cells (5×10^4 cells/well) plus actinomycin D (final concentration 1 μg/well). Recombinant human TNF (Cetus Corp., Emeryville, CA) was used as a positive control in this assay. The cells were incubated at 37°C for 18 h, supernatants were discarded, and the remaining viable adherent cells stained. The absorbance of each well was read at 540 nm with a MicroELISA autoreader. Units of TNF were defined as the reciprocal of the dilution at which 50% cytotoxicity occurs.

Antibodies and Immunohistochemistry

Antimurine TNFα was produced by immunization of rabbits with recombinant murine TNFα administered in multiple intradermal sites with complete Freund's adjuvant. Anti-IL-1α and -β were prepared by similar immunization of a goat and a rabbit, respectively, with recombinant murine IL-1α or IL-1β (Pfizer Central Research, Dept. Molecular Genetics, Groton, CT). These antisera reacted with appropriate proteins in Western blot analysis. In competitive inhibition experiments, to demonstrate antibody specificity, immunostaining for murine TNFα, IL-1α, and β showed nearly 100% inhibition by exogenous addition of respective recombinant cytokines with virtually no cross-reactivity. Immunolocalization studies were performed as follows. Macrophage monolayers were fixed for 5 min in 4% paraformaldehyde in PBS and rinsed twice with PBS. Prior to staining they were fixed for 3 min in absolute methanol. The slides were rinsed again with PBS and then treated with a 3% hydrogen peroxide solution to inactivate any remaining peroxidase activity. The slides were next blocked with a 1:50 dilution of normal goat or rabbit serum, then decanted and exposed to dilutions of anti-TNFα (1:1,000), anti-IL-1α (1:2,000), anti-IL-1β (1:1,000) or a similar dilution of control serum. After 10 min incubation at 37°C, the slides were rinsed, overlaid with biotinylated goat antirabbit IgG (1:200) or rabbit antigoat IgG (1:200) (Vector Laboratories, Burlingame, CA) and incubated, followed by three additional rinses with PBS. The slides were next treated with peroxidase-labeled streptavidin (Sigma), incubated again, rinsed thrice, then overlaid with substrate chromogen (3-amino 9-ethyl carbazole) for 5 min at 37°C to allow for color development. Mayer's hematoxylin was used as a counterstain.

RESULTS

Kinetics of IL-1α, IL-1β, and TNFα Gene Transcription

In order to determine optimal times to examine the effects of PGE_2 on mRNA synthesis, we initially estab-

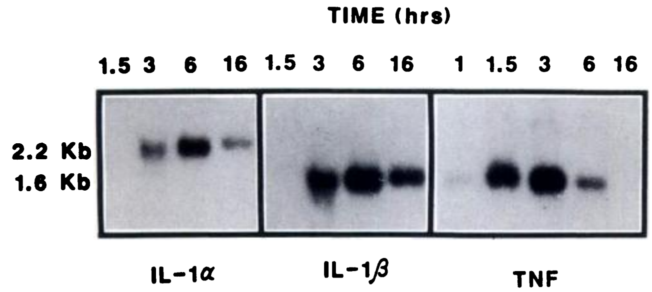

Fig. 1. Kinetics of mRNA accumulation in LPS-stimulated (1 μg/ml) CFA macrophages for murine IL-1α, IL-1β, and TNFα as assessed by Northern blot analysis.

TABLE 1. Dose-Dependent Suppression of Cell-Associated Monokine Bioactivities

	Suppression of cell-associated bioactivity (%)[a]	
PGE_2 (M)	TNF	IL-1
5×10^{-10}	0	0
5×10^{-9}	15 ± 5	0
5×10^{-8}	37 ± 8	0
5×10^{-7}	74 ± 10	2 ± 7
5×10^{-6}	95 ± 5	4 ± 6

[a]Macrophages were challenged with 1.0 μg/ml LPS in the presence or absence of graded concentrations of PGE_2. After 4 h (time of maximal cell-associated TNF activity) or 6 h (time of maximal cell-associated IL-1 activity), the monolayers were washed thrice, scraped from their dishes, and assayed after freeze thawing in 1 ml of culture medium. Values are mean ± SEM of three determinations.

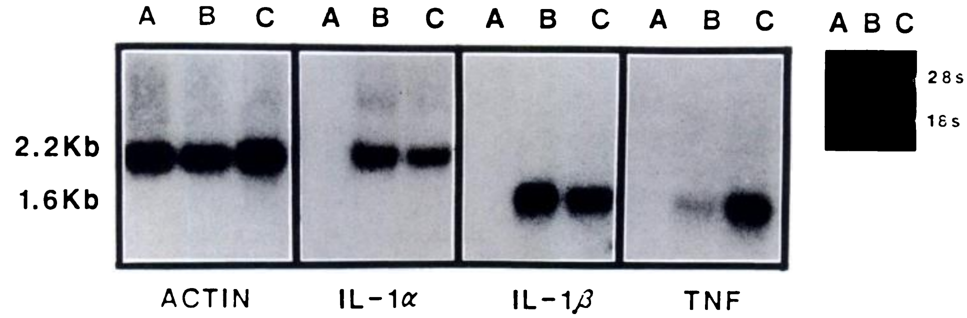

Fig. 2. Northern blot analysis of mRNA accumulation for actin, IL-1α, IL-1β, and TNFα in CFA macrophages treated with: A) control media; B) 1 μg/ml LPS + PGE_2 10^{-7}M; or C) 1 μg/ml LPS. Macrophage TNF mRNA was assessed 3 h post-treatment, and IL-1 was assessed 6 h post-treatment.

lished the kinetics of IL-1 and TNF mRNA accumulation following LPS stimulation. As shown in Figure 1, IL-1α and IL-1β displayed similar patterns of mRNA accumulation, with peak message expression occurring at 6 h poststimulation. Message for TNFα achieved maximal levels sooner, about 3 h poststimulation. These kinetics paralleled the accumulation of the respective bioactive materials detected in cell lysates (data not shown).

Regulation of IL-1α, IL-1β, and TNFα by Prostaglandin E_2

We and others have previously reported that PGE_2 will dose-dependently inhibit the production of IL-1 and TNF activity in stimulated macrophage supernatants [8–11]. Using similar concentrations of PGE_2, we found no effect on cell lysate IL-1 activity, yet PGE_2 was effective in suppressing TNF (Table 1). The results suggest that PGE_2 affected IL-1 and TNF synthesis and release by different mechanisms.

To further examine the apparent differential regulation of TNF and IL-1 by PGE_2, we performed Northern blot analysis on mRNA isolated at 3 and 6 h, respectively, from stimulated CFA macrophages that were treated or untreated with PGE_2. As shown in Figure 2, the accumulation of actin mRNA was unaffected by LPS or PGE_2, suggesting that these treatments did not non-specifically affect global mRNA production. Prostaglandin E_2 at 10^{-6} M had no effect on the accumulation of LPS-induced mRNA for IL-1α or β. In contrast, the maximal accumulation of TNF mRNA at 3 h was markedly suppressed by PGE_2 (greater than 50% as assessed by laser densitometry), as compared to the LPS-stimulated control.

As shown in Figure 3, immunohistochemical localization of IL-1 and TNF confirmed the bioassay experiments. Cellular expression of TNFα, IL-1α, and β was clearly induced by LPS. Interestingly, PGE_2 had no effect on cell-associated IL-1α or IL-1β expression. Conversely, TNF expression was significantly suppressed by 1 μM PGE_2. The staining patterns for IL-1α and -β are suggestive of a diffuse cytoplasmic localization of these proteins, whereas the staining for TNFα appears to be more localized within the cytoplasm (see arrows, Figure 3f). These distinct staining patterns may reflect differences in the packaging and cellular processing of these monokines.

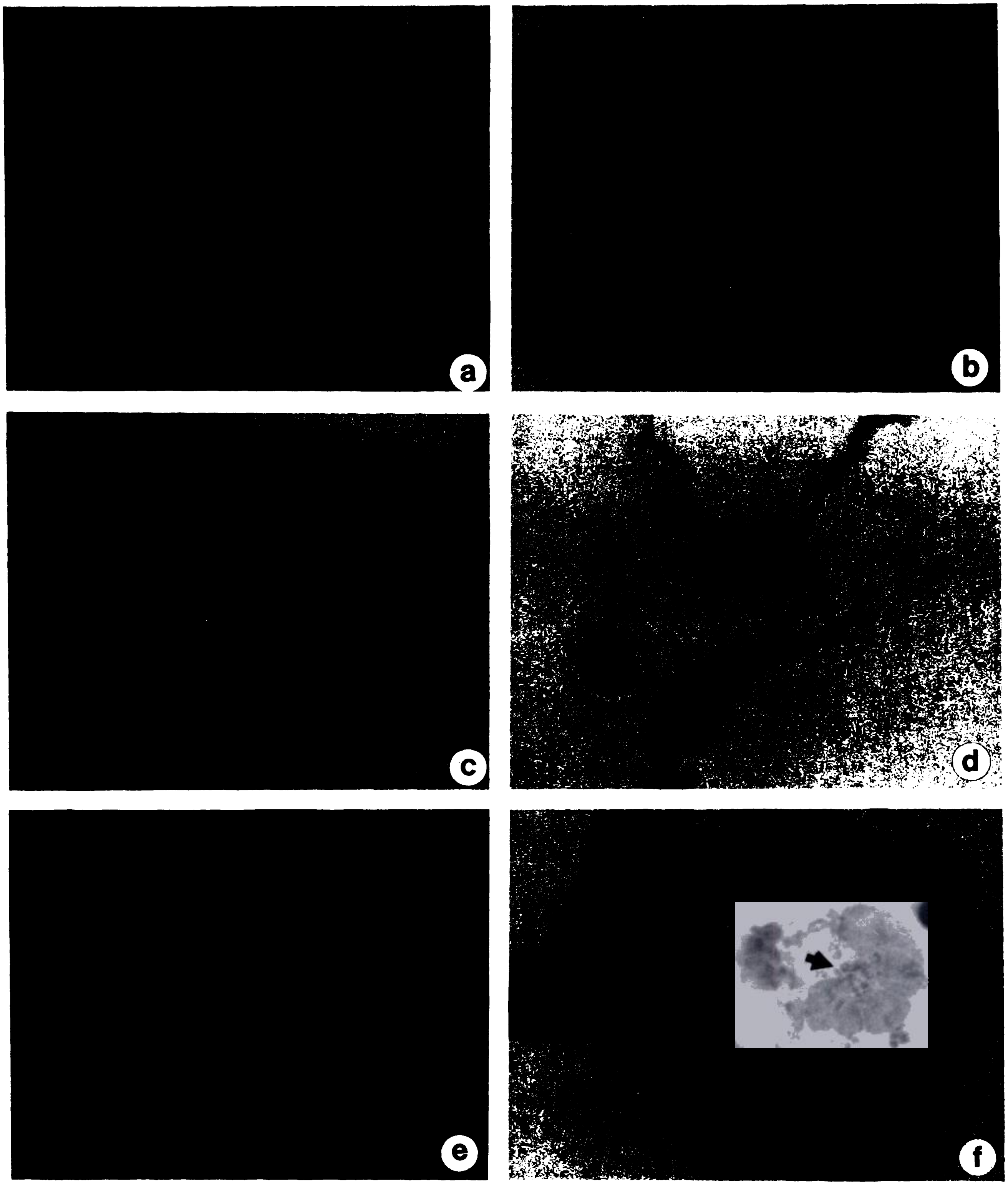

Fig. 3. Effect of PGE_2 on cell-associated monokine expression detected immunohistochemically. Cells were treated for 6 h with 1 µg/ml LPS in the presence or absence of PGE_2 (1×10^{-6}M). The monolayers were then fixed and stained for monokine expression by immunolocalization. a: Anti-IL-1α, (PGE_2 + LPS). b: Anti-IL-1α, (LPS). c: Anti-IL-1β, (PGE_2 + LPS). d: Anti-IL-1β, (LPS). e: Anti-TNFα, (PGE_2 + LPS). f: Anti-TNFα, (LPS).

DISCUSSION

We and others have reported suppression of macrophage IL-1 and TNF release by PGE_2 [8–11]. In the present study, we examined the effect of PGE_2 on the production of monokine mRNA and the accumulation of monokine activity in cell lysates. Northern blot analysis demonstrated no effect of PGE_2 on IL-1α or -β mRNA accumulation, yet TNF mRNA was markedly reduced. These data suggest that PGE_2 affected TNF production at the level of transcription, whereas any affect on IL-1 activity would seem to be post-transcriptional. We have recently further substantiated (data not shown) this notion by nuclear transcription analyses, showing no effect of PGE_2 on genomic IL-1α mRNA, yet significant reductions in genomic mRNA for TNF [22]. These results do not preclude the possibility of post-transcriptional effects of PGE_2 on TNF synthesis.

Determination of monokine activities in cell lysates revealed that both IL-1 and TNF activities were detectable. Prostaglandin E_2 had different effects on these activities, causing nearly complete suppression of TNF but not IL-1 activity. Other investigators have reported both cytosolic and membrane-associated IL-1 activity in activated macrophage populations [23–27]. Preliminary studies in our laboratory in which stimulated cells have been separated into membrane and cytosolic components suggest that the bulk of cell-associated IL-1 bioactivity is cytosolic, with a much smaller membrane component. This membrane component, although minimal, may represent an important functional component. As yet we have not specifically determined the effect of PGE_2 on membrane associated IL-1 activity.

Our immunohistochemical studies lend additional support to the bioassay data. These studies dramatically demonstrated that PGE_2 caused a suppression of TNF but not IL-1α or -β expression in macrophages. The patterns of staining suggested that TNF and IL-1 are distributed differently within cells, such that TNF is localized and IL-1 is dispersed in cytoplasm. The diffuse nature of IL-1 staining agrees with the ultrastructural studies of Singer et al. indicating that IL-1 is located in cytoplasmic ground substance of human monocytes [28].

Interleukin-1 and tumor necrosis factor share a number of biological activities. Though similar, these cytokines act via discrete receptors on target cells, and share no apparent structural homology. The results of this study imply that the regulation of these two cytokines by a potential endogenous autokine, prostaglandin E_2, is quite distinct. PGE_2 exerts a profound inhibition on TNF at the transcriptional and possibly post-transcriptional level. In contrast, the previously reported PGE_2 mediated suppression of IL-1 activity in macrophage cultures appears to be a post-translational event. The differential regulation of these cytokines may have implications as to the roles of these mediators in health and disease.

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