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Interleukin 1- and tumor necrosis factor-stimulation of prostaglandin E2 synthesis in MDCK cells, and potentiation of this effect by cycloheximide

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The effects of interleukin (IL)-1α, IL-1β and TNFα on prostaglandin-E2 synthesis in Madin-Darby canine kidney (MDCK) cells were investigated. IL-1β time- and dose-dependently stimulated prostaglandin-E2 synthesis. While TNFα produced a comparatively small but significant stimulation of PGE2 release, coincubation of IL-1β with TNFα produced a marked synergistic stimulation of PGE2 release. The effect of IL-1β and of IL-1β and TNFα was apparent as early as after 2 h of incubation. The enhanced PGE2 synthesis was inhibited by indomethacin as well as actinomycin D, while cycloheximide surprisingly potentiated PGE2 synthesis in response to both IL-1β and TNFα. IL-1α alone was ineffective in stimulating a significant release of PGE2 at concentrations as high as 10 nM. However, it also showed a marked synergistic interaction with TNFα in stimulating PGE2 release.

Interleukin-1; TNFα; Prostaglandin-E2; Madin-Darby canine kidney cell; Cycloheximide

1. INTRODUCTION

It is well established that IL-1 plays an important role in the mediation of inflammatory responses, being released mainly by stimulated macrophages and exerting a range of effects on many different cell types [1-3]. One of the effects exerted by IL-1 is the stimulation of prostaglandin synthesis. Released prostaglandin may be an important effector in inflammation and pathological development in diseased kidney states. Markedly increased arachidonic acid metabolism is found in several models of renal disease [4]. Studies have shown a natriuretic effect of IL-1 in rats that is inhibitable by prior administration of indomethacin [5]. Stimulated PGE2 release by IL-1 has also been demonstrated in rat renal mesangial cells [6,7]. TNFα is also well known to mediate inflammatory responses, sharing many of IL-1's effects and often acting synergistically with IL-1 in vitro and in vivo in inducing various responses, including prostaglandin synthesis [8]. In this paper, we report IL-1- and TNFα-induced PGE2 synthesis in Madin-Darby canine kidney (MDCK) cells, a kidney epithelial cell line with many characteristics of distal tubule cells [9].

2. MATERIALS AND METHODS

2.1. *Cell culture*

MDCK cells from the American Type Culture Collection in passages 93-100 were used and grown in medium consisting of DMEM/HAM's F12 (1:1), 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, penicillin 50 U/ml, and streptomycin 50 μg/ml.

2.2. *Incubation of cells*

Cells were plated in 16 mm diameter wells and used for experiments when confluent. Cells were washed twice with PBS and incubated with DMEM containing 0.1 mg/ml of fatty acid-free bovine serum albumin (Sigma) with or without agents. At the indicated times, medium was removed and stored at −20°C until determination of PGE2 by radioimmunoassay (New England Nuclear).

2.3. *Cytoxicity*

A possible cytotoxic effect of the tested compounds was checked with a colorimetric assay. The uptake of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into MDCK cells and its modification to a colored product by living cells was measured as described [10].

2.4. *Chemicals*

r-IL-1α (>10^7 U/mg) was obtained from Biogen, Geneva. r-IL-β (>10^7 U/ml) was from Biotechnology Department of Ciba-Geigy, Basel, Switzerland. rTNFα (>2×10^7 U/mg) was obtained from Boehringer Mannheim, FRG. Cycloheximide, actinomycin D and MTT were obtained from Sigma, and indomethacin was obtained from Ciba-Geigy, Basel.

3. RESULTS

Cells incubated for 24 h with 1 nM IL-1β showed a more than 5-fold increase in PGE2 release over control cells (fig.1). Cells incubated with IL-1α at the same concentration did not show a significant increase in PGE2 release. Incubation with 1 nM TNFα resulted in a moderate 1.9-fold increase. Coincubation of 1 nM IL-1β with 1 nM TNFα dramatically boosted PGE2 release by over 20-fold, while coincubation of 1 nM IL-1α and 1 nM TNFα produced a nearly 5-fold increase in PGE2 release.

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Abbreviations IL-1, interleukin-1; TNF, tumor necrosis factor; PGE2, prostaglandin E2

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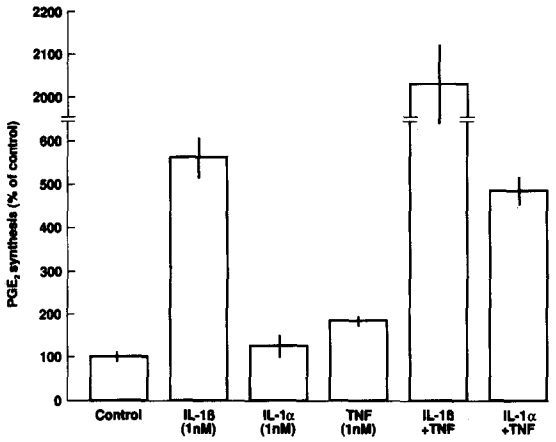

Fig.1. Effect of different cytokines on PGE2 synthesis in MDCK cells. PGE2 synthesis in control cells was 42 ± 2 pg/10^6 cells. Values are means from 4 experiments ± SE.

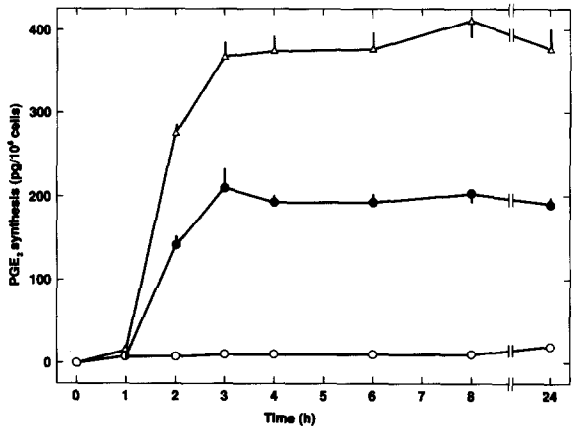

Fig.3. Time-course of cytokine-stimulated PGE2 synthesis in MDCK cells. Cells were stimulated with (○), vehicle; (●), IL-1β (1 nM); (△), IL-1β (1 nM) + TNFα (1 nM). Values are means from 4 experiments ± SE.

IL-1β and TNFα alone did not show significant stimulation of PGE2 release at concentrations below 1 nM (fig.2), while increasing the concentration from 1 nM to 10 nM, the highest concentration tested, produced an increased stimulation of PGE2 synthesis. IL-1α, which was ineffective at 1 nM, also showed no clearly significant stimulation of PGE2 release at 10 nM (data not shown).

A time-course was performed on cells incubated with IL-1β (1 nM) or IL-1β (1 nM) and TNFα (1 nM) (fig.3). No significant stimulation of PGE2 release was seen during the first hour. PGE2 was released between the 1- and 3-h time points, after which a plateau was reached that lasted for the remainder of the 24-h incubation.

The cyclooxygenase inhibitor indomethacin, at a concentration of 200 nM, expectedly inhibited stimulation of PGE2 synthesis when incubated simultaneously with IL-1β (1 nM), TNFα (1 nM), or the combination (fig.4). The inhibitor of transcription actinomycin D, at a concentration of 10 μM, also effectively maintained PGE2 release at or near control values, which, along with the initial time lag in PGE2 release, indicates the involvement of de novo protein synthesis in the stimulation of PGE2 synthesis. However, when the cytokines were coincubated for 24 h with the translation inhibitor cycloheximide at a concentration of 10 μM, there was a marked augmentation of PGE2 release. This effect was especially dramatic with TNFα, with cycloheximide boosting PGE2 release more than 50-fold over that induced by TNFα alone. A time-course of combined TNFα and cycloheximide stimulation of PGE2 synthesis revealed a biphasic effect, with a relatively small synergistic increase at 4 h that plateaued for at least 4 h, and a dramatic increase between 8 and 24 h (fig.5).

It is known that cycloheximide can sensitize some cells which are normally resistant to TNFα-cytotoxicity [11]. To test for a possible cytotoxic effect, an MTT incorporation assay was performed following a 24-h incubation with different agents. Combined incubation

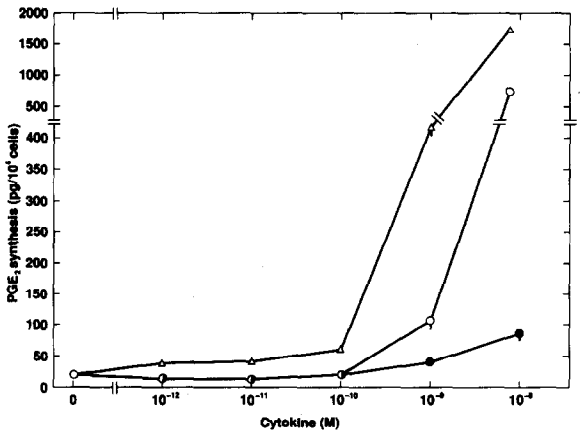

Fig.2. Dose dependency of different cytokines on PGE2 synthesis in MDCK cells. Cells were stimulated for 24 h with (○), IL-1β; (●), TNFα; (△), IL-1β and TNFα. Values are means from 4 experiments ± SE.

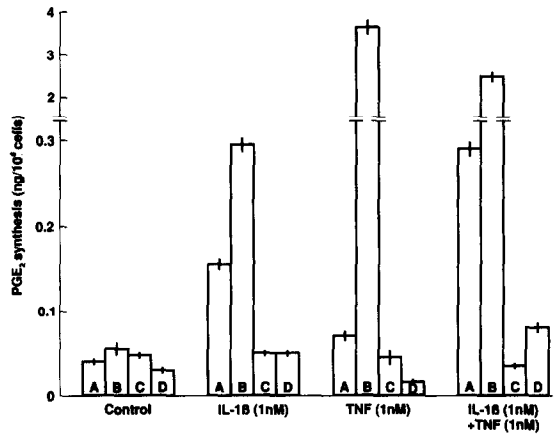

Fig.4. Effect of different compounds on cytokine-stimulated PGE2 synthesis in MDCK cells. Cells were incubated for 24 h with the indicated concentrations of cytokines + (A), vehicle; (B), cycloheximide (10 μM); (C), actinomycin D (10 μM); (D), indomethacin (200 nM). Values are means from 4 experiments ± SE.

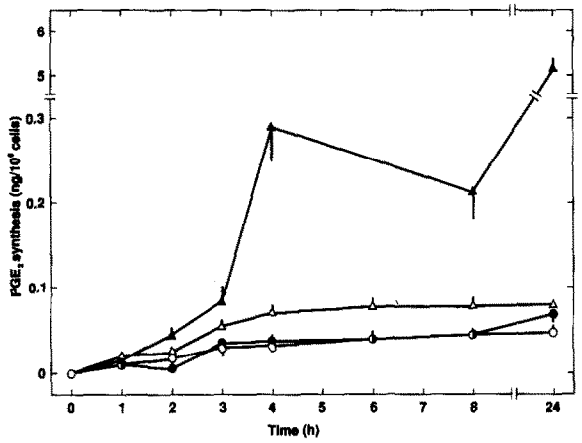

Fig.5. Time-course of cycloheximide-induced potentiation of TNFα-stimulated PGE2 synthesis in MDCK cells. Cells were incubated for the indicated time periods with (○), vehicle; (●), cycloheximide (10 μM); (△), TNFα (1 nM); (▲), cycloheximide (10 μM) + TNFα (1 nM). Values are means from 4 experiments ± SE.

with 10 μM cycloheximide and 1 nM TNFα resulted in a 70% drop in cell survival, whereas neither agent alone exhibited cytotoxicity. In a further experiment, the cyclooxygenase inhibitor indomethacin was coincubated with TNFα and cycloheximide in order to determine whether prostaglandin synthesis is required for the cytotoxic effect. As has been reported with other cell types by others [12,13], indomethacin did not protect the cells from the cytotoxicity of TNFα and cycloheximide (data not shown). These results suggest that the large stimulation of PGE2 synthesis by cycloheximide and TNFα is a consequence of cell injury, although a separate parallel mechanism cannot be ruled out. An MTT incorporation assay was also performed following a 24-h incubation with IL-1β (1 nM) and cycloheximide (10 μM). Contrary to the findings with TNFα, no significant cytotoxicity was observed. Cell death can therefore probably be excluded as an explanation for the moderate potentiation of IL-1β-stimulated PGE2 synthesis by cycloheximide.

4. DISCUSSION

The present study demonstrates a time- and dose-dependent stimulation of PGE2 synthesis in MDCK cells by IL-1β, low-level stimulation by TNFα, and a marked synergistic stimulation by IL-1β and TNFα and by IL-1α and TNFα. These findings of IL-1- and TNFα-stimulated PGE2 synthesis are similar to those found in renal mesangial cells, where a synergistic interaction was also found, and IL-1β was found to be much more potent than IL-1α [7]. However, the time-course is different in the present study, with PGE2 synthesis occurring early and over a relatively short period, compared with the 8-h time lag and prolonged synthesis found in mesangial cells. In addition, the cycloheximide potentiation of the stimulation of PGE2 synthesis by both IL-1β and TNFα is a new finding. Renal eicosanoids in general have been shown to affect such processes as renal blood flow, renin release, and water and ion reabsorption [4]. PGE2 in particular is important for its role as a vasodilator. PGE2 has also been shown to modulate cAMP levels in many cells, including MDCK cells [9,14], which may in turn negatively regulate PGE2 synthesis [15]. Secreted PGE2 has been suggested to feedback on IL-1 production by invasive macrophages [4,16], and there is also evidence that it suppresses macrophage cytotoxicity [17] and Ia antigen expression [18].

IL-1- and TNF-stimulation of PGE2 in MDCK cells, in addition to representing a response to macrophage invasion in the inflamed kidney, may also be relevant to local regulation of PGE2 synthesis by neighboring cells under normal physiological conditions, as many cell types other than macrophages, including renal mesangial cells [19,20], have been shown to secrete IL-1 and TNFα.

The stimulation of PGE2 synthesis is most likely attributable to an induction of cyclooxygenase and/or phospholipase A2. IL-1 has been shown to stimulate cyclooxygenase synthesis in fibroblasts [21,22] and increase intracellular phospholipase A2 in rabbit chondrocytes [23]. Similarly, TNFα has been shown to induce phospholipase A2 and a phospholipase-activating protein in endothelial cells [24]. The effect of cycloheximide in potentiating the stimulation of PGE2 by IL-1β and by TNFα was unexpected. The very large increase in PGE2 synthesis during a 24-h incubation with both TNFα and cycloheximide is most likely a consequence of cell injury following cycloheximide-induced susceptibility to TNFα cytotoxicity by an as yet incompletely determined mechanism [15,25,26].

However, a smaller increase in PGE2 synthesis observable at 4 h, after which a plateau is reached for at least 4 h, suggests that another mechanism of potentiation of PGE2 synthesis may be operating at earlier time points. Consistent with this suggestion of a second mechanism is the observation of a doubling in IL-1β-stimulated PGE2 synthesis by coincubation with cycloheximide (fig.4), which is not due to a cytotoxic effect. The explanation for this result is not entirely clear, but may involve a labile repressor protein whose disappearance allows a greater stimulation by IL-1β and TNFα of the transcription of a cyclooxygenase or phospholipase A2 gene. Cycloheximide-stimulation of mRNA synthesis has been reported for several genes, including the human β-interferon genes [27], mouse actin genes [28], and immunoglobulin k-light-chain genes [29], and the stimulatory effect of cycloheximide has in several cases been traced to specific *cis*-regulatory elements [30–32], although the regulatory proteins involved are far more elusive.

Negative transcriptional regulation by trans-acting factors has been shown to play an important part in

eukaryotic gene regulation [33,34], and the present findings suggest that such regulation may also play a role in the control of prostaglandin synthesis, at least in certain tissues. Measurement of cyclooxygenase and phospholipase A2 mRNA levels would be an important next step in elucidating the effect of cycloheximide on IL-1β- and TNFα-stimulated PGE2 synthesis.

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