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Tumor necrosis factor stimulates prostaglandin production and cyclic AMP levels in rat cultured mesangial cells

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Human recombinant tumor necrosis factor-α (TNF) was found to stimulate the production of prostaglandins (PG) by cultured rat mesangial cells. This effect was demonstrable from 6 h, was dose dependent and affected the synthesis of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$. It required both RNA and protein synthesis but was not associated with a modification of cell proliferation. TNF also stimulated adenosine 3′-5′ cyclic monophosphate (cAMP) levels in the mesangial cell culture medium. Indomethacin suppressed the effect of TNF on PGs but only reduced that on cAMP, indicating that PG production partly mediates the increase in cAMP. These findings demonstrate that mesangial cells can be a target for TNF and that the mechanism of TNF action includes stimulation of both PG production and cAMP levels.

Tumor necrosis factor; Prostaglandin; Adenosine cyclic monophosphate; Glomerular mesangium

1. INTRODUCTION

Tumor necrosis factor is a monocyte/macrophage-derived protein which induces a variety of effects on cell functions, including modulation of proliferation rate, production of interleukin 1 (IL-1), PGE_2, and collagenase, as well as induction of surface antigens [1]. Because infiltration of glomeruli by monocytes/macrophages is associated with local production of TNF in nephrotoxic serum nephritis [2,3], the question arises whether TNF could interact with glomerular cells and modify their functions. In this model of experimental glomerulonephritis, mesangial cell proliferation [4] and enhanced PG production [5] have been shown to occur following glomerular macrophage accumulation. Moreover, local PGE_2 synthesis results in an increase of the cAMP content of glomeruli [6], thereby inducing a fall in the ultrafiltration coefficient [7]. We therefore elected to determine the effects of human recombinant TNF on the proliferation rate of cultured rat masangial cells and on their synthesis of both cAMP and PGs, including PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, the stable end-product of PGI_2, which are the main metabolites of the cyclooxygenase pathway in these cells [8].

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2. MATERIALS AND METHODS

2.1. *Mesangial cell culture*

Mesangial cells were cultured from isolated rat glomeruli according to [9]. Briefly, glomeruli were prepared from the renal cortices of Sprague-Dawley rats by sieving techniques and differential centrifugations. After treatment by 300 U/ml of collagenase (300 U/mg; Sigma, St. Louis, MO) for 30 min at 37°C, glomeruli were resuspended in RPMI 1640 medium (Flow Laboratories, Irvine, UK), buffered with 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes) to pH 7.4, and supplemented with 10% decomplemented fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM glutamine. Then, they were plated onto plastic culture multidish wells (Costar, Cambridge, MA) and cultured at 37°C in 5% CO_2 in air. Under such conditions, mesangial cells appeared after 7–14 days in culture and reached confluency by day 21. Cells were studied after 15–21 days of culture.

The effects of TNF on PG and cAMP synthesis by mesangial cells were determined after addition of human recombinant

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TNF (2×10^7 U/mg; Genzyme, Boston, MA) to the culture medium (1 ml) of 12 multidish wells containing 150000–200000 cells. When necessary, actinomycin D, cycloheximide, or indomethacin (Sigma) were added together with TNF.

2.2. *Determination of PG synthesis by mesangial cells*

Radioimmunoassay of PGs was carried out in the culture medium of mesangial cells after extraction according to [10]. PGs were assayed at four increasing dilutions. [^{3}H]PGE_2, [^{3}H]$PGF_{2\alpha}$ and [^{3}H]6-keto-$PGF_{1\alpha}$ were purchased from the Radiochemical Center (Amersham, UK). Anti-PGE_2 antibody was obtained from Institut Pasteur (Paris), anti-6-keto-$PGF_{1\alpha}$ antibody was a gift from Dr Dunn (Cleveland, OH) and anti-$PGF_{1\alpha}$ antibody had been raised in the laboratory. These antibodies cross-react only slightly with other PGs and could be considered as specific [11].

2.3. *Determination of cyclic AMP levels in the mesangial cell culture medium*

At the end of incubation, 0.2 ml of culture medium was removed, and added to 0.4 ml of an ice-cold ethanol/formic acid mixture (85:15, v/v) in order to extract cAMP. After 30 min at 4°C, extracts were evaporated under nitrogen and the dry residues were resuspended in the radioimmunoassay buffer. Radioimmunoassay of cAMP was performed after acetylation of the samples, as described [6].

2.4. *Statistical analysis*

The results are presented as means ± SE. Where appropriate, degrees of statistical significance were determined by the paired Student *t*-test.

3. RESULTS

Different concentrations of TNF were added to the culture medium, and rat mesangial cells were incubated for 48 h (fig.1a). Maximal stimulation of PGE_2 production was observed after incubation with 100 ng/ml. To determine whether increased PGE_2 synthesis resulted from a change in cell number, cell counts were also performed. Treatment with TNF for 48 h did not result in an increase of cell number at any of the concentrations used (fig.1a). When the kinetics of PGE_2 synthesis in response to TNF were examined, an increase first became apparent by 6 h after addition of TNF (100 ng/ml) and progressive additional increases occurred between 9 and 48 h (fig.1b). A decline in PGE_2 production was then observed both in the presence and the absence of TNF. Similarly, TNF elicited a rise in the production of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (fig.2). Maximum effects of TNF also occurred at 100 ng/ml and for a 48-h incubation period, but the percent increases above con-

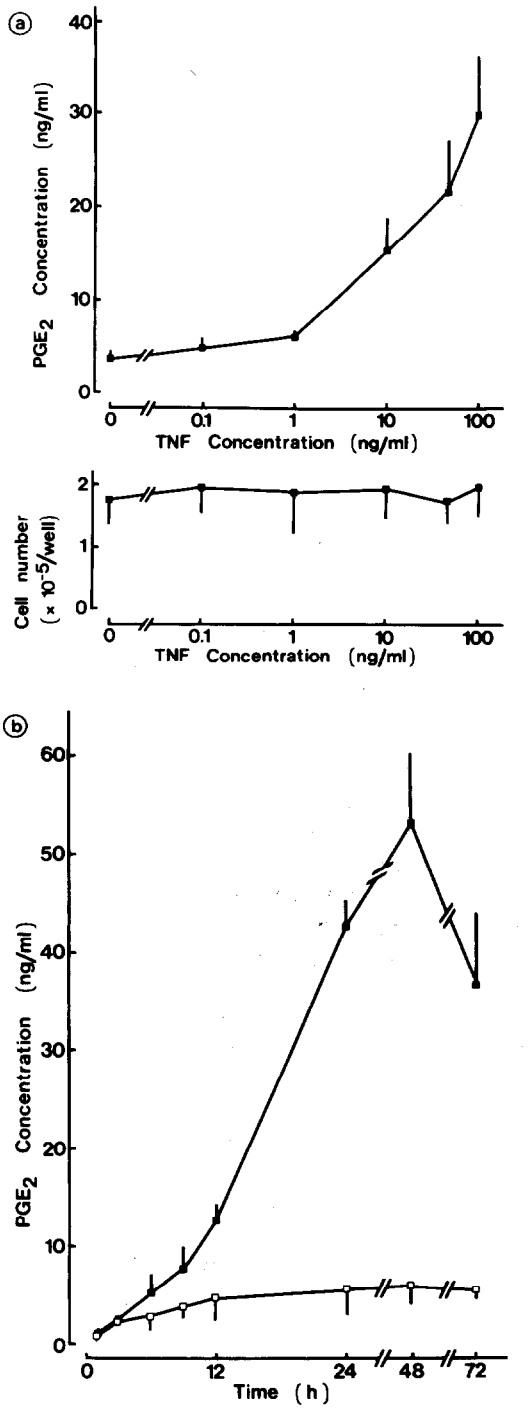

Fig.1. Effect of TNF on PGE_2 synthesis (upper part) and cell growth (lower part). Incubations were carried out for 48 h (a). Time course of PGE_2 release from mesangial cells incubated without (□) and with (■) 100 ng/ml TNF (b). Means and SE of values obtained in 4–5 experiments are given.

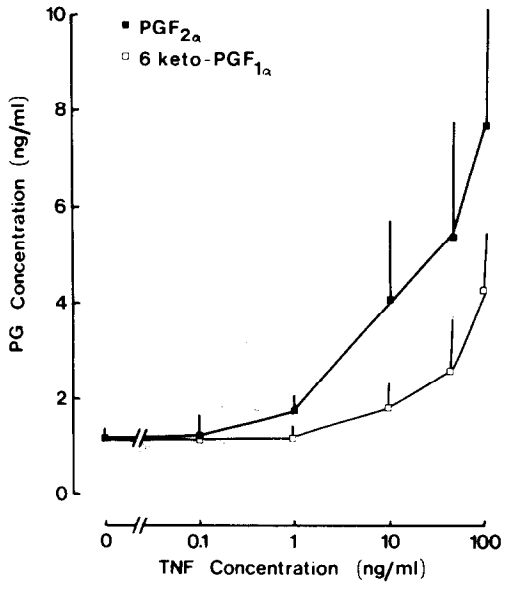

Fig.2. Effect of TNF on $PGF_{2\alpha}$ (■) and 6-keto-$PGF_{1\alpha}$ (□) synthesis. Incubations were carried out for 48 h. Means and SE of values obtained in 3 experiments are given.

trol levels were slightly lower for $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (6.5- and 3.5-fold control values, respectively) than for PGE_2. Cell treatment by 0.1 μg/ml actinomycin D, which inhibits RNA synthesis, did not significantly decrease basal PGE_2 production, but reduced the stimulatory effect of 100 ng/ml TNF by 80.8 ± 7.2% ($n = 4$, $P < 0.05$). In addition, the stimulatory effect of TNF was inhibited by 60.6 ± 4.6% in the presence of 0.1 μg/ml cycloheximide, an inhibitor of protein synthesis ($n = 4$, $P < 0.05$) (table 1). The time course of cAMP concentration in the medium in the presence of TNF is shown in fig.3. A slight increase in cAMP concentration was detectable after 9–12 h of TNF stimulation. This was followed by a major peak of stimulation at 48 h which represented a 2-fold increase. To determine whether the observed effects were related to the increased production of PGs, studies utilizing a cyclooxygenase inhibitor (indomethacin, 10 μM) were also carried out. Addition of indomethacin reduced by 90% PGE_2 production by mesangial cells under basal conditions, and completely counteracted the stimulatory effect of TNF (table 2). In parallel, indomethacin treatment caused a marked decrease of the basal values of cAMP, as well as a reduction of cAMP accumulation in response to TNF from +0.822 ± 0.037 to +0.139 ± 0.014 pmol/ml ($n = 3$, $P < 0.005$). However, the TNF-induced increase in cAMP levels remained statistically significant ($P < 0.01$).

Table 1

Effect of actinomycin D and cycloheximide on PGE_2 synthesis by mesangial cells

Addition	PGE_2 synthesis (ng/ml)	
	Control	TNF (100 ng/ml)
None	5.73 ± 2.14	35.62 ± 6.20
Actinomycin D (0.1 μg/ml)	3.90 ± 0.94	6.34 ± 0.82*
Cycloheximide (0.1 μg/ml)	7.38 ± 2.85	16.51 ± 5.75*

Mesangial cells were incubated for 48 h. The resulting mesangial supernatant was collected and assayed for PGE_2 concentration. Results are expressed as means ± SE of 4 experiments. * $P < 0.05$, compared with values obtained without addition of drug

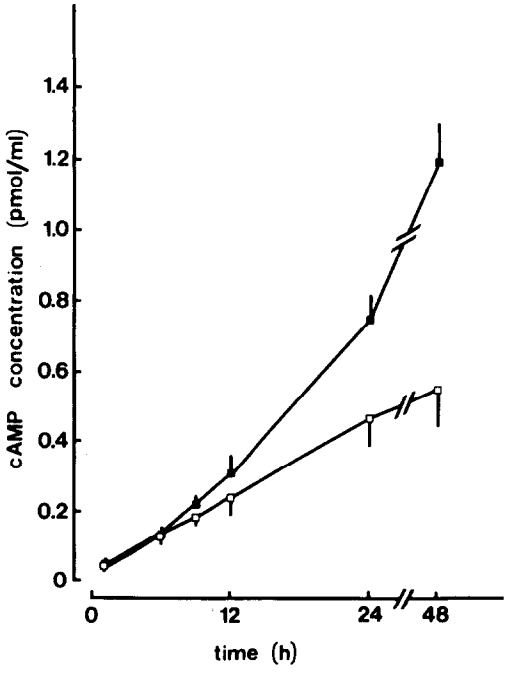

Fig.3. Time course of cAMP concentration in the culture medium of rat mesangial cells incubated with (■) and without (□) 100 ng/ml TNF. Means and SE of values obtained in 3 experiments are given.

Table 2

Effect of indomethacin on PGE_2 synthesis by mesangial cells and cAMP levels in their culture medium

Addition	PGE_2 synthesis (ng/ml)		cAMP concentration (pmol/ml)	
	Control	TNF (100 ng/ml)	Control	TNF (100 ng/ml)
None	5.94 ± 1.37	30.52 ± 5.08	0.642 ± 0.049	1.464 ± 0.082
Indomethacin (10 μM)	0.52 ± 0.09	0.43 ± 0.06	0.145 ± 0.066	0.283 ± 0.073

Mesangial cells were incubated for 48 h. The resulting mesangial supernatant was collected and assayed for both PGE_2 and cAMP concentrations. Results are expressed as means ± SE of 3 experiments, each performed in duplicate

4. DISCUSSION

Recent studies have demonstrated that TNF and IL-1, although being biochemically and immunologically distinct proteins, share a number of similar biological activities [1]. In nephrotoxic serum nephritis, both TNF and IL-1 are released in glomeruli [2,3]. In contrast with the effects of IL-1, those of TNF on glomerular cells have never been reported. Our results demonstrate, for the first time, that recombinant human TNF stimulates PG and cAMP production by rat mesangial cells in culture. The stimulatory effect of TNF was dose dependent and affected the production of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, which are the major metabolites of the cyclooxygenase pathway in these cells [8]. A similar stimulation of PGE_2 and PGI_2 secretion has been observed previously in mesangial cells exposed to IL-1 for 24 h [12]. However, the time course of PG production which we observed in TNF-stimulated mesangial cells was not identical to that reported for mesangial cells stimulated by IL-1. After 1 h of incubation with IL-1, there was a significant increase in the production of PGE_2 but a decrease in that of PGI_2 whereas such a period of exposure to TNF was too short to alter the synthesis of these two PGs. The stimulatory effect of TNF on PG production thus required a long incubation period, as observed previously in other cell types including fibroblasts [13,14], resting macrophages [15] and osteoblast-like cells [16]. Such a lag-time suggests that RNA and protein synthesis are probably involved in the process of stimulating the cellular production of PGs. This hypothesis is supported in our study by the fact that actinomycin D and cycloheximide reduced the effect of TNF (table 1). Because TNF affects the generation of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, TNF-induced protein(s) would interfere with either cyclooxygenase or phospholipase activities.

In mesangial cells incubated with TNF, the enhanced production of PGs was associated with an increase of cAMP concentration. These two events are linked since: (1) the time courses of PGE_2 and cAMP accumulations exhibited similar patterns (fig.3); and (2) indomethacin completely abolished PGE_2 production and reduced cAMP accumulation in response to TNF (table 2). However, a small but significant increase in cAMP was observed, even when TNF-induced PGE_2 synthesis was suppressed by indomethacin, suggesting that other mechanisms are involved in this effect.

Stimulations of PG production by mesangial cells incubated with TNF and of cAMP levels in their culture medium were not the result of alterations in cell number (fig.1). In fact, the mitogenic activity of TNF depends on the cell type since TNF appears to stimulate the growth of human fibroblasts [1] but not of osteoblast-like cells [16] and even to reduce the proliferation rate of endothelial cells [17]. However, TNF can affect the growth of mesangial cells under experimental conditions different from those we used, in particular when the cells are cultured in serum-free medium (not shown).

In order to relate the significance of our observations to in vivo situations it is necessary to estimate if the amount of TNF generated by monocytes/macrophages in glomeruli during nephrotoxic serum nephritis is sufficient to affect mesangial PG and cAMP syntheses. Wiggins et al. [3] have reported that the concentration of TNF released by glomeruli isolated on day 14 of this ex-

perimental model reached 410 U/ml per 1000 glomeruli, which corresponds to a concentration of 20 ng/ml of recombinant TNF in our study. Since in vitro TNF effects were obtained for concentrations between 1 and 100 ng/ml, the amount of TNF released by infiltrating macrophages at this stage of nephrotoxic serum nephritis is sufficient to increase the local production of PGs and concentration of cAMP.

Our present finding that TNF stimulates PG production by cultured mesangial cells and cAMP in their culture medium suggests that this cytokine released together with IL-1 from inflammatory cells may play a pivotal role in modulating mesangial cell function in the course of glomerulonephritis. The finding that TNF stimulates cAMP levels, in part independently of its effect on PG production, suggests that this cytokine could act directly on cAMP synthesis or catabolism.

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