Interleukin 1 and tumor necrosis factor stimulate cGMP formation in rat renal mesangial cells

Josef Pfeilschifter and Heidi Schwarzenbach

Ciba-Geigy Ltd, Research Department, Pharmaceuticals Division, R-1056.P.23, CH-4002 Basel, Switzerland

Received 5 September 1990

Treatment of mesangial cells with recombinant human interleukin 1β (IL-1β) or recombinant human tumor necrosis factor α (TNFα) dose-dependently increased cGMP formation. Both IL-1β and TNFα-stimulated formation of cGMP occurred after a initial lag period of 4 to 8 hours. Treatment of cells with actinomycin D, cycloheximide or dexamethason completely abolished cytokine-induced cGMP formation. Furthermore, the guanylate cyclase inhibitor Methylene blue completely blocked IL-1β- and TNFα-stimulated cGMP generation. N^G-mono-methyl-L-arginine attenuated IL-1β- and TNFα-induced cGMP production, an effect that was reversed by L-arginine.

Interleukin 1; Tumor necrosis factor; cGMP; Guanylate cyclase; Nitric oxid; Mesangial cell

1. INTRODUCTION

IL-1 and TNFα are polypeptide hormones produced mainly by macrophages and play a central role in the pathogenesis of acute glomerular injury and nephritis [1]. Proliferation of mesangial cells is a hallmark of progressive glomerular disease and IL-1 and TNFα synthesized by mesangial cells may serve to perpetuate proliferation in an autocrine manner [2,3]. Furthermore, IL-1 stimulates the release of a specific type IV collagenase [4] and both cytokines, IL-1 and TNFα, enhance the synthesis and release of phospholipase A_2 and prostaglandins from mesangial cells [5].

The signalling pathways triggered by IL-1 and TNFα are far from clear, for there are a number of reports, each suggesting a different second messenger system. In mesangial cells IL-1 and TNFα have no direct effect on phosphoinositide hydrolysis and Ca^{2+} mobilization ([6], J. Pfeilschifter and M. Ochsner, unpublished results) or on cAMP generation formation [7,8]. Recently, Kester and colleagues reported that IL-1 activates a phosphatidylethanolamine-degrading phospholipase C in mesangial cells [6]. In the present study, we have investigated the effects of IL-1β and TNFα on cGMP formation in mesangial cells. The present data suggest that the NO-producing L-arginine pathway is activated in mesangial cells following administration of IL-1β or TNFα with a subsequent increase in cellular cGMP levels.

Correspondence address: J. Pfeilschifter, Ciba-Geigy Ltd, R-1056. P. 23, CH-4002 Basel, Switzerland

Abbreviations: IL-1, interleukin 1; TNF, tumor necrosis factor; IBMX, 3-isobutyl 1-methylxanthine; NMMA, N^G-monomethyl-L-arginine

2. MATERIALS AND METHODS

2.1. *Chemicals*

Recombinant human IL-1β ($>10^7$ U/ml) was prepared by the Biotechnology Department of Ciba-Geigy Ltd, Basel, Switzerland; recombinant human TNFα ($>10^7$ U/ml) was obtained from Boehringer Mannheim; FRG, Methylene blue, L-arginine, N^G-monomethyl-L-arginine acetate (NMMA), cyclo-heximide, actinomycin D and dexamethason were obtained from Sigma, St. Louis, MO; all cell culture nutrients were from Boehringer Mannheim, FRG; all other chemicals were from Merck, Darmstadt, FRG.

2.2. *Cell culture*

Rat renal mesangial cells were cultivated as described previously [9]. The cells were grown in RPMI 1640 supplemented with 20% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and bovine insulin at 0.66 U/ml (Sigma). Mesangial cells were characterized morphologically by phase contrast microscopy, positive staining for the intermediate filaments desmin and vimentin, which is considered to be specific for myogenic cells [10], negative staining for factor VIII related antigen and cytokeratin, excluding endothelial and epithelial contaminations, respectively.

2.3. *Incubation of cells and cGMP determination*

Confluent mesangial cells in 35 mm diameter dishes were preincubated with IL-1β or TNFα together with the different inhibitors for the indicated time periods. Thereafter, the medium was removed and the cells were rinsed twice and incubated for an additional 10 min, either in the presence or absence of the phosphodiesterase inhibitor 3-isobutyl 1-methylxanthine (IBMX) (0.5 mM). The reaction was terminated by aspiration of the medium and addition of 1 ml ice-cold 5% (w/v) trichloroacetic acid. For extraction of cGMP the dishes were put on ice for 1 h. The trichloroacetic acid was then removed by 3 extractions with 10× volume of water-saturated diethyl ether, and cGMP was determined by radioimmunoassay (New England Nuclear).

3. RESULTS

Short-term incubations of mesangial cells with IL-1β or TNFα for 20, 40, and 60 min did not increase cellular

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

cGMP levels, irrespective of whether phosphodiesterase was blocked by IBMX or not (data not shown). In a next step, cells were preincubated with IL-1β or TNFα for 24 h and cGMP formation was determined. Basal levels of cGMP in control cells were 0.34 ± 0.04 pmol/mg of protein and treatment with IL-1β (1 nM) or TNFα (1 nM) increased these values to 5.3 ± 0.4 and 1.0 ± 0.3 pmol/mg of protein, respectively (values are means ± SD, $n = 4$). In the presence of IBMX (0.5 mM) for a 10 min period after cytokine treatment, the effects of both IL-1β and TNFα were greatly enhanced (Table 1). This indicates that the effect of Il-1β and TNFα on cGMP were due to stimulation of guanylate cyclase rather than to inhibition of cGMP degradation by phosphodiesterase. Further evidence for this suggestion is provided by the complete inhibition of cytokine-induced cGMP formation by the guanylate cyclase inhibitor Methylene blue (Table II). As shown in Table I IL-1β- and TNFα-stimulated formation of cGMP was dose-dependent with a significant stimulation occurring at 10 pM. The induction of cGMP formation by both cytokines requires a lag time of 4 to 8 h as shown in Fig. 1. Co-incubation with actinomycin D (10 μM), cycloheximide (10 μM) or dexamethasone (1 μM) completely suppressed IL-1β- or TNFα-stimulated cGMP formation (Table II), indicating that an increase in both transcription and translation is involved. Co-incubation with NMMA (10 μM) attenuated IL-1β- and TNFα-stimulated cGMP formation as shown in Table II. L-arginine (2 mM) added together with NMMA counteracted the effect of NMMA (Table II), thus suggesting that an NO-like substance is involved in cytokine-induced cGMP formation in mesangial cells.

Table I

Dose-dependence of IL-1β- and TNFα-stimulated cGMP formation in mesangial cells

Addition	cGMP (pmol/mg of protein)
Control	1.8 ± 0.2
IL-1β 1 pM	2.3 ± 0.4
10 pM	5.8 ± 0.7
100 pM	52.0 ± 9.8
1 nM	434 ± 160
10 nM	392 ± 32
TNFα 1 pM	2.0 ± 0.4
10 pM	9.4 ± 2.2
100 pM	21.0 ± 1.2
1 nM	41.2 ± 3.7
10 nM	169 ± 28

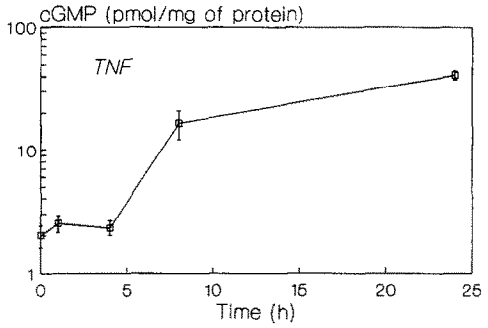
Mesangial cells were preincubated for 24 h with the indicated concentrations of IL-1β or TNFα. Thereafter, the medium was removed and the cells were incubated for an additional 10 min in the presence of IBMX (0.5 mM) and cGMP was determined as described in section 2. Results are means ± SD for 4 experiments

Table II

Effect of different compounds on IL-1β- and TNFα-stimulated cGMP formation in mesangial cells

Addition	cGMP (pmol/mg of protein)
Control	6.7 ± 1.2
IL-1β	275 ± 63
IL-1β + NMMA (10 μM)	136 ± 23
IL-1β + NMMA (10 μM) + L-arginine (2 mM)	235 ± 24
Il-1β + Methylene blue (50 μM)	0.4 ± 0.3
IL-1β + actinomycin D (10 μM)	6.8 ± 0.6
IL-1β + cycloheximide (10 μM)	10.1 ± 0.2
IL-1β + dexamethason (1 μM)	12.5 ± 2.3
TNF	37.8 ± 7.8
TNF + NMMA (10 μM)	23.9 ± 2.7
TNF + NMMA (10 μM) + L-arginine (2 mM)	35.1 ± 3.1
TNF + Methylene blue (50 μM)	0.3 ± 0.1
TNF + actinomycin D (10 μM)	1.5 ± 0.4
TNF + cycloheximide (10 μM)	3.1 ± 0.3
TNF + dexamethason (1 μM)	4.0 ± 1.0

Mesangial cells were preincubated for 24 h with IL-1β (1 nM) or TNFα (1 nM) together with the indicated drugs. Thereafter, the medium was removed and the cells were incubated for an additional 10 min in the presence of IBMX (0.5 mM) and cGMP was determined as described in Section 2. Results are means ± SD for 4 experiments



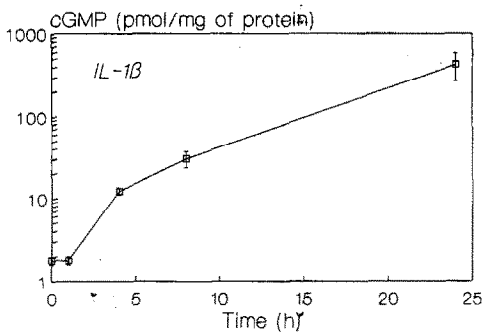


Fig. 1. Time-course of IL-1β- and TNFα-stimulated cGMP formation in mesangial cells. Mesangial cells were preincubated with IL-1β (1 nM) or TNFα (1 nM) for 24 h. Thereafter, the medium was removed and the cells were incubated for an addition 10 min in the presence of IBMX (0.5 mM) and cGMP was determined as described in section 2. Results are means ± SD for 4 experiments.

4. DISCUSSION

Mesangial cells are a major determinant in the regulation of the glomerular filtration rate [11]. Mor-

phologically, mesangial cells resemble vascular smooth muscle cells and are able to contract upon stimulation by vasoactive hormones like angiotensin II (see [12,13] for review). The interaction of infiltrating monocytes and intrinsic mesangial cells plays an important role in the pathogenesis of certain forms of immune-mediated glomerulonephritis [1]. The release of IL-1 or TNFα by infiltrating monocytes promotes mesangial cell proliferation [2,3], secretion of phospholipase A_2 and prostanoids [5], as well as secretion of a neutral proteinase activity [4]. These events may contribute to the morphological changes observed in progressive glomerular diseases.

To our knowledge, this is the first report demonstrating that IL-1β and TNFα potently increase cGMP levels in mesangial cells. The lag period of 4 to 8 h, as well as the inhibitory effects of actinomycin D, cycloheximide and dexamethason indicate that both cytokines induce the synthesis of a protein that may be responsible for the chronic stimulation of guanylate cyclase activity in mesangial cells. The inhibitory effect of NMMA, a competitive inhibitor of nitric oxide synthase and its reversal by L-arginine suggest that IL-1β and TNFα stimulate the synthesis of a NO-like intermediate in mesangial cells which increases cGMP by activating soluble guanylate cyclase. This suggestion is further strengthened by the complete inhibition of cytokine-evoked cGMP generation by Methylene blue, an inhibitor of soluble guanylate cyclase. Lipopolysaccharide preparations which are well known stimulators of cytokine production [14] have been shown to stimulate cGMP formation in rat liver [15] and spleen cells [16]. As in mesangial cells, a lag period of 2 h was required and cGMP accumulation was blocked by actinomycin D and cycloheximide. Furthermore, lipolysaccharide- and interferon γ-stimulated murine macrophages secrete NO [17].

Elevations of mesangial cell cGMP levels cause relaxation and result in an increased glomerular filtration rate [18]. However, it has been observed that the afferent arteriole and glomeruli are contracted in endotoxemia [19] and these events are accompanied by a fall in glomerular filtration rate [20]. Circulating products of Gram negative bacteria are trigger substances that initiate these conditions in endotoxemia by stimulating the release of cytokines such as IL-1 or TNF [14]. A possible explanation for these contradictory results may be that IL-1 and TNF also modulate other signalling pathways that are involved in the regulation of the contractile state of mesangial cells such as phosphoinositide turnover and adenylate cyclase activity (Pfeilschifter and Schwarzenbach, unpublished results). The cross-communication between these signalling cascades will determine the final outcome in the cells and thus influence the functional parameter of increased or decreased cell contraction. Further experiments will be necessary to investigate the importance of cytokine-induced NO formation in mesangial cells for the fall in glomerular filtration rate with subsequent development of acute renal failure.

REFERENCES

[1] Wardle, E.N. (1988) Nephron 49, 265–276.
[2] Lovett, D.H., Ryan, J.L. and Sterzel, R.B. (1983) J. Immunol. 131, 2830–2836.
[3] Melcion, C., Lachman, L., Killen, P.D., Morel-Maroger, L. and Striker, G.E. (1982) Transplant. Proc. 14, 559–564.
[4] Martin, J., Lovett, D.H., Gemsa, D., Sterzel, R.B. and Davies, M. (1986) J. Immunol. 137, 525–529.
[5] Pfeilschifter, J., Pignat, W., Märki, F. and Vosbeck, K. (1989) Biochem. Biophys. Res. Commun. 159, 385–394.
[6] Kester, M., Simonson, M.S., Mené, P. and Sedor, J.R. (1989) J. Clin. Invest. 83, 718–723.
[7] Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F. and Vosbeck, K. (1990) Fresenius J. Anal. Chem. 337, 139–140.
[8] Pfeilschifter, J., Leighton, J., Pignat, W., Märki, F. and Vosbeck, K. (1990) Biochem. J. (in press).
[9] Pfeilschifter, J., Kurtz, A. and Bauer, C. (1984) Biochem. J. 223, 855–859.
[10] Travo, P., Weber, K. and Osborn, M. (1982) Exp. Cell Res. 139, 87–94.
[11] Dworkin, L.D., Ichikawa, I. and Brenner, B.M. (1983) Am. J. Physiol. 244, F95–F104.
[12] Pfeilschifter, J. (1989) Eur. J. Clin. Invest. 19, 347–361.
[13] Mené, P., Simonson, M.S. and Dunn, M.J. (1989) Physiol. Rev. 69, 1347–1424.
[14] Dinarello, C.A. (1989) Advanc. Immunol. 44, 153–205.
[15] Graber, S.E. and Clancey, M.A. (1985) Proc. Soc. Exp. Biol. Med. 180, 163–169.
[16] Bomboy, J.D. and Graber, S.E. (1980) J. Lab. Clin. Med. 95, 654–659.
[17] Stuehr, D.J., Gross, S.S., Sakuma, I., Levi, R. and Nathan, C.F. (1989) J. Exp. Med. 169, 1011–1020.
[18] Singhal, P.C., DeCandido, S., Satriano, J.A., Schlondorff, D. and Hays, R.M. (1989) Am. J. Physiol. 257, C86–C93.
[19] Conger, J.D., Falk, S.A. and Guggenheim, S.J. (1981) J. Clin. Invest. 67, 1334–1346.
[20] Tolins, J.P., Vercellotti, G.M., Wilkowske, M., Ha, B., Jacob, H.S. and Raji, L. (1989) J. Lab. Clin. Med. 113, 316–324.