Transforming growth factor β_2 differentially modulates interleukin- 1β - and tumour-necrosis-factor- α -stimulated phospholipase A_2 and prostaglandin E_2 synthesis in rat renal mesangial cells

Josef PFEILSCHIFTER,* Werner PIGNAT, Jonathon LEIGHTON, Fritz MÄRKI, Klaus VOSBECK and Sefik ALKAN

Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland

Treatment of rat glomerular mesangial cells with transforming growth factor β_2 ($TGF\beta_2$) stimulates prostaglandin E_2 (PGE_2) synthesis. Actinomycin D, cycloheximide and diclofenac attenuate the $TGF\beta_2$ -induced PGE_2 formation. As shown previously, two proinflammatory cytokines, interleukin 1β ($IL-1\beta$) and tumour necrosis factor α ($TNF\alpha$), are potent stimuli for PGE_2 and phospholipase A_2 secretion from mesangial cells. We report here that, whereas $TGF\beta_2$ potentiates the $IL-1\beta$ - and $TNF\alpha$ -evoked PGE_2 production, it strongly inhibits the phospholipase A_2 secretion induced by both cytokines. In addition, the inhibitory effect of $TGF\beta_2$ on phospholipase A_2 secretion is not due to the augmented PGE_2 formation.

INTRODUCTION

The role of monocytes and macrophages as effector cells in the pathogenesis of acute glomerular injury and nephritis is well documented (for review see [1]). We and others have previously demonstrated that secretory products of macrophages, such as interleukin 1 ($IL-1$) and tumour necrosis factor α ($TNF\alpha$), stimulate prostaglandin E_2 (PGE_2) [2–4] and phospholipase A_2 (PLA_2) [4] synthesis in mesangial cells. Furthermore, $IL-1$ and $TNF\alpha$ synergistically interact to induce release of PGE_2 as well as PLA_2 by mesangial cells [4,5]. Extracellular PLA_2 is pro-inflammatory and vasoactive, and mediates hyperaemia and a marked inflammatory reaction when injected intracutaneously into rabbits [6] or intra-articularly into rats [7]. Thus secretion of PLA_2 by mesangial cells may play a role in the propagation of inflammatory reactions within the glomerulus. In contrast, the synthesis of vasodilatory PGE_2 may serve to maintain glomerular filtration rate and renal plasma flow during inflammation [8,9]. Another potentially important secretory product of macrophages is transforming growth factor β ($TGF\beta$), which exerts diverse actions on many cells, ranging from growth promotion to growth inhibition and immunomodulation (for review see [10]). $TGF\beta$ has been purified from normal kidney [11], and the presence of high-affinity receptors for $TGF\beta$ on mesangial cells has been reported [12]. $TGF\beta$ augments the production of extracellular-matrix proteins, in particular collagen and fibronectin, by mesangial cells [12], and inhibits the proliferation of the cells [12–14]. Recently, the presence of large quantities of $TGF\beta$ in chronic inflammatory conditions has been demonstrated [10]. Therefore it was decided to investigate the effects of $TGF\beta$ on mesangial cells which are stimulated with inflammatory cytokines, such as $IL-1\beta$ and $TNF\alpha$. We report here that, whereas $TGF\beta_2$ inhibits PLA_2 secretion, it potentiates the synthesis of PGE_2 stimulated by $IL-1\beta$ and $TNF\alpha$.

MATERIALS AND METHODS

Cell culture

Rat mesangial cells were cultivated as described previously [15]. The cells were grown in RPMI 1640 supplemented with

20% (v/v) fetal-calf serum, penicillin (100 units/ml), streptomycin (100 μ g/ml) and bovine insulin at 0.66 unit/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 [16], negative staining for Factor VIII-related antigen and cytokeratin, excluding endothelial and epithelial contaminations respectively. For these experiments, passages 12–35 of mesangial cells were used.

Incubation of cells and prostaglandin analysis

Confluent mesangial cells cultured in 16 mm-diameter wells were washed twice with phosphate-buffered saline [17] and incubated with 1 ml of Dulbecco's modified Eagle's medium [18] containing 0.1 mg of fatty-acid-free BSA (Sigma)/ml with or without the different agents. After the indicated time periods, the medium was withdrawn and centrifuged in an IEC Centra-7R laboratory centrifuge. The supernatant was removed and frozen in liquid nitrogen, and stored at -80°C until assay of PLA_2 and PGE_2 . PGE_2 was determined by radioimmunoassay (New England Nuclear). Cells were dissolved in 0.5 M-NaOH and protein was determined by the method of Lowry *et al.* [19], with BSA as a standard.

PLA_2 assay

PLA_2 activity was determined with [$1-^{14}\text{C}$]oleate-labelled *Escherichia coli* as substrate as described in ref. [20]. The substrate was prepared by growing *E. coli* in the presence of [$1-^{14}\text{C}$]oleate, followed by autoclaving to inactivate endogenous phospholipases. More than 95% of the label incorporated by *E. coli* was in phospholipid and, as demonstrated by hydrolysis with snake venom (*Crotalus adamanteus*), more than 95% of the [$1-^{14}\text{C}$]oleate was in the *sn*-2 position of the phospholipids [20]. Assay mixtures (1.0 ml) contained 100 mM-Tris/HCl buffer (pH 7.0), 1.0 mM- CaCl_2 , 2.5×10^8 [$1-^{14}\text{C}$]oleate-labelled *E. coli* cells (5 nmol of phospholipid, 5000–8000 c.p.m.) and the enzyme to be tested at a dilution producing 10–15% substrate hydrolysis. Reaction mixtures were incubated for 1 h at 37°C in a shaking water bath. The reaction was stopped by the addition of 5 ml of

Abbreviations used: $TGF\beta_2$, transforming growth factor β_2 ; $IL-1\beta$, interleukin 1β ; $TNF\alpha$, tumour necrosis factor α ; PLA_2 , phospholipase A_2 ; PGE_2 , prostaglandin E_2 ; IC_{50} , half-maximal inhibitory concentration.

* To whom correspondence and reprint requests should be addressed.

propan-2-ol/n-heptane/1 M-H₂SO₄ (40:10:1, by vol.), followed by 2 ml of heptane and 3 ml of water. After vigorous shaking and phase separation, a portion (2.5 ml) of the upper phase was passed over a column of silicic acid (3.5 cm × 0.5 cm). Free [1-¹⁴C]oleic acid was quantitatively eluted with 1 ml of ethyl acetate. Radioactivity was determined in a scintillation counter. PLA₂ activity is expressed as [1-¹⁴C]oleate radioactivity released by 100 µl of cell culture supernatant.

Chemicals

[1-¹⁴C]Oleic acid was purchased from Amersham International, Amersham, Bucks., U.K. Pig TGFβ₂ was obtained from AMS Biotechnology Ltd., Zürich, Switzerland; its activity was tested in an ovalbumin-specific mouse T-cell proliferation assay [21]. It inhibited the T-cell proliferation with an IC₅₀ of 0.05 ng/ml (S. Alkan, unpublished work). Recombinant human IL-1β, indomethacin and diclofenac were prepared by Ciba-Geigy Ltd., Basel, Switzerland; recombinant human TNFα was from Boehringer, Mannheim, Germany; actinomycin D and cycloheximide were from Sigma, St. Louis, MO, U.S.A.

Statistics

Statistical analysis was by Student's *t* test, and *P* < 0.05 was used as the criterion for statistical significance.

RESULTS

TGFβ₂ stimulates PGE₂ synthesis in mesangial cells

The addition of TGFβ₂ to mesangial cells caused a dose-dependent stimulation of PGE₂ synthesis, as shown in Fig. 1(b). Significant effects were seen at 1 ng of TGFβ₂/ml, and a 5-fold increase in the production of PGE₂ was noted at 10 ng/ml. This time-dependent event occurred after an initial lag period of approx. 8 h (Fig. 1a). Simultaneous addition of actinomycin D (10 µM) or cycloheximide (10 µM) attenuated the TGFβ₂-induced PGE₂ formation, thus demonstrating that transcription and protein synthesis are necessary for enhanced PGE₂ synthesis (Table 1). The potent cyclo-oxygenase inhibitor diclofenac totally abolished the TGFβ₂-stimulated PGE₂ production, as shown in Table 1.

TGFβ₂ inhibits basal PLA₂ release from mesangial cells

We have recently shown that mesangial cells secrete low amounts of a Ca²⁺-dependent PLA₂ [4]. Addition of TGFβ₂ caused a significant attenuation of this basal PLA₂ release from the cells, as shown in Fig. 1. TGFβ₂ at 10 pg/ml already significantly decreased PLA₂ secretion, and at 10 ng/ml approx. 50 % decrease was observed. There was a lag period of 8 h before this inhibitory activity of TGFβ₂ became evident (Fig. 1a).

TGFβ₂ differentially modulates cytokine-stimulated PGE₂ and PLA₂ secretion from mesangial cells

We have previously shown that treatment of mesangial cells with IL-1α, IL-1β or TNFα induced PGE₂ synthesis and the release of PLA₂ activity. When given together, TNFα interacted in a synergistic fashion with IL-1α and IL-1β, to augment both PGE₂ synthesis and PLA₂ secretion [4]. As we have observed, TGFβ₂ itself has effects on PGE₂ and PLA₂ release from mesangial cells. Thus we were interested to see whether TGFβ₂ also modulates the stimulatory effects of IL-1β and TNFα. Cells were treated for 24 h with IL-1β (1 nM) or TNFα (1 nM) together with different concentrations of TGFβ₂, and the total release of PGE₂ and PLA₂ was determined. The results presented in Fig. 2 clearly demonstrate that TGFβ₂ differentially modulated IL-1β- and TNFα-stimulated eicosanoid synthesis and enzyme formation. TGFβ₂ dose-dependently potentiated PGE₂ synthesis

Fig. 1. Time course (a) and dose-dependence (b) of TGFβ₂ effects on PGE₂ and PLA₂ release from mesangial cells

Confluent mesangial cells were incubated with TGFβ₂ (1 ng/ml) (●, ▲) or vehicle (○, △) for the indicated time periods (a) or with the indicated concentrations of TGFβ₂ for 24 h (b). Thereafter, PGE₂ (○, ●) and PLA₂ (△, ▲) were determined in the medium as outlined in the Materials and methods section. Results are means ± S.E.M. (*n* = 4).

Table 1. Effect of different compounds on TGFβ₂-stimulated PGE₂ synthesis in mesangial cells

Confluent mesangial cells were incubated with actinomycin D (10 µM), cycloheximide (10 µM) or diclofenac (100 nM) alone or together with TGFβ₂ (1 ng/ml) as indicated, for 24 h. After the incubation period the medium was removed and PGE₂ was determined as outlined in the Materials and methods section. Results are means ± S.E.M. (*n* = 4).

Addition	PGE ₂ (ng/mg of protein)
Control	0.73 ± 0.05
TGFβ ₂	2.82 ± 0.41
Actinomycin D	0.45 ± 0.10
Cycloheximide	0.64 ± 0.08
Diclofenac	0.44 ± 0.04
TGFβ ₂ + actinomycin D	1.13 ± 0.04
TGFβ ₂ + cycloheximide	1.04 ± 0.14
TGFβ ₂ + diclofenac	0.54 ± 0.04

in response to both cytokines, with significant effects at 10 pg/ml (Fig. 2a). In contrast, TGFβ₂ attenuated IL-1β- and TNFα-stimulated PLA₂ secretion, with significant effects at 100 pg/ml (Fig. 2b).

TGFβ₂ inhibition of PLA₂ secretion is not mediated by prostaglandins

Recently it was reported that TGFβ-induced stimulation of PGE₂ synthesis in lung fibroblasts may act as an autoregulatory feedback loop to limit the effects of TGFβ on connective-tissue protein synthesis [22]. To investigate such a possibility in our cell-culture model, we determined the effect of the potent cyclo-oxygenase inhibitor indomethacin on cytokine-induced PGE₂ and PLA₂ secretion from mesangial cells. Treatment of the cells with indomethacin (300 nM) totally suppressed the cytokine-induced PGE₂ production. In contrast, indomethacin had no effect whatsoever on IL-1β-mediated PLA₂ secretion and the inhibitory action of TGFβ₂ on this PLA₂ release (results not shown). Similar results were obtained for TNFα and TGFβ₂, where indomethacin fully blocked PGE₂ synthesis, without

Fig. 2. Dose-dependence of TGF β_2 effects on IL-1 β - and TNF α -stimulated PGE₂ and PLA₂ release from mesangial cells

Confluent mesangial cells were incubated for 24 h with IL-1 β (1 nM) (○, ●), TNF α (1 nM) (△, ▲) or vehicle (□, ■) together with the indicated concentrations of TGF β_2 . After the incubation period, the medium was withdrawn and PGE₂ (a: ○, △, □) and PLA₂ (b: ●, ▲, ■) were determined as outlined in the Materials and methods section. Results are means $\pm$ S.E.M. ($n = 4$).

having any effect on PLA₂ secretion (results not shown). These results unambiguously exclude PGE₂ as the mediator of the inhibitory action of TGF β_2 on PLA₂ formation and release from mesangial cells.

DISCUSSION

The TGF β family of molecules are small homo- and heterodimeric polypeptides originally purified from platelets [23], placenta [24] and kidney [11]. These multifunctional molecules are produced by neoplastic and normal cells, including macrophages and lymphocytes (for review see [10]). In the kidney, besides the presence of TGF β , the existence of high-affinity TGF β receptors on glomerular endothelial, epithelial and mesangial cells has been reported [12]. TGF β increased the production of extracellular-matrix components, especially of collagen and fibronectin by mesangial cells [12]. Furthermore, TGF β was shown to inhibit mesangial cell proliferation in response to growth-factor stimulation [12–14]. From these results it is tempting to speculate that TGF β may play an important role in the modulation of processes that critically regulate the initiation of progressive glomerular diseases.

Our results presented here clearly show that TGF β_2 differentially modulates PGE₂ and PLA₂ secretion from mesangial cells under basal conditions, as well as in response to proinflammatory cytokines, such as IL-1 β and TNF α . TGF β_2 itself was a weak stimulator of PGE₂ synthesis, with threshold concentrations of 1 ng/ml. However, TGF β_2 potently synergized with IL-1 β and TNF α in stimulating PGE₂ production already at 10 pg/ml (Figs. 1 and 2). In contrast, TGF β_2 inhibited basal and cytokine-stimulated PLA₂ release from mesangial cells (Figs. 1 and 2). Although indomethacin treatment completely suppressed TGF β_2 - and IL-1 β - or TNF α -stimulated PGE₂ formation, there was no effect of indomethacin on TGF β_2 -induced inhibition of PLA₂ secretion. These data strongly argue against an auto-regulatory feedback mechanism exerted by PGE₂, as has been reported for TGF β -stimulated collagen and fibronectin formation in fibroblasts [22]. The ability of TGF β to augment certain cytokine-induced events, while dampening others, is well documented. TGF β has been shown to inhibit IL-1-mediated protease

release and cartilage proteoglycan degradation when added to rabbit chondrocytes [25]. Furthermore, the combined action of TGF β and IL-1 α resulted in a synergistic increase in PGE production in rheumatoid synovial cells [26]. But, to our knowledge, this is the first report on two IL-1 β - and TNF α -induced functional responses in the same cell type that are differentially modulated by TGF β_2 .

The newly discovered dual action of TGF β_2 might have some physiological significance, namely that TGF β_2 inhibits cytokine-stimulated release of the proinflammatory and destructive enzyme PLA₂ from mesangial cells, and on the other hand TGF β_2 potentiates the synthesis of vasodilatory PGE₂, which serves to maintain glomerular functions during inflammation. These observations suggest that TGF β_2 may function as a regulatory signal in the kidney, which protects the organ from damage resulting from chronic immunological or inflammatory reactions.

We gratefully acknowledge the technical assistance of Danièle Kaemmerlen.

REFERENCES

- Wardle, E. N. (1988) *Nephron* **49**, 265–276
- Lovett, M. D., Resch, K. & Gemsa, D. (1987) *Am. J. Pathol.* **129**, 543–551
- Baud, L., Perez, J., Friedlander, G. & Ardaillou, R. (1988) *FEBS Lett.* **239**, 50–54
- Pfeilschifter, J., Pignat, W., Vosbeck, K. & Märki, F. (1989) *Biochem. Biophys. Res. Commun.* **159**, 385–394
- Topley, N., Floege, J., Wessel, K., Hass, R., Radeke, H. H., Kaever, V. & Resch, K. (1989) *J. Immunol.* **143**, 1989–1995
- Pruzanski, W., Vadas, P. & Fornasier, V. (1986) *J. Invest. Dermatol.* **86**, 380–383
- Vadas, P., Pruzanski, W., Kim, J. & Fornasier, V. (1989) *Am. J. Pathol.* **134**, 807–811
- Rahman, M. A., Stork, J. E. & Dunn, M. J. (1987) *Kidney Int.* **32**, Suppl. **22**, S40–S48
- Dworkin, L. D., Ichikawa, I. & Brenner, B. M. (1983) *Am. J. Physiol.* **244**, F95–F104
- Wahl, S. M., McCartney-Francis, N. & Mergenhagen, S. E. (1989) *Immunol. Today* **10**, 258–261
- Roberts, A. B., Anzano, M. A., Meyers, C. A., Wideman, J., Blacher, R., Pan, Y.-C. E., Stein, S., Lehrman, S. R., Smith, J. M., Lamb, L. C. & Sporn, M. B. (1983) *Biochemistry* **22**, 5692–5698
- MacKay, K., Striker, L. J., Stauffer, J. W., Doi, T., Agodola, L. Y. & Striker, G. E. (1989) *J. Clin. Invest.* **83**, 1160–1167
- Jaffer, F., Saunders, C., Shultz, P., Throckmorton, D., Weinsell, E. & Abboud, H. E. (1989) *Am. J. Pathol.* **135**, 261–269
- Yamashita, W., MacCarthy, E. P., Hsu, A., Gartside, P. S. & Ooi, B. S. (1989) *Clin. Exp. Immunol.* **77**, 285–288
- Pfeilschifter, J., Kurtz, A. & Bauer, C. (1984) *Biochem. J.* **223**, 855–859
- Travo, P., Weber, K. & Osborn, M. (1982) *Exp. Cell Res.* **139**, 87–94
- Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182
- Smith, J. D., Freeman, G., Vogt, M. & Dulbecco, R. (1960) *Virology* **12**, 185–196
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Märki, F. & Franson, R. (1986) *Biochim. Biophys. Acta* **879**, 149–156
- Alkan, S. S. (1978) *Eur. J. Immunol.* **8**, 112–118
- Diaz, A., Varga, J. & Jimenez, S. A. (1989) *J. Biol. Chem.* **264**, 11554–11557
- Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D. M. & Sporn, M. B. (1983) *J. Biol. Chem.* **258**, 7155–7160
- Frolik, C. A., Dart, L. L., Meyers, C. A., Smith, D. M. & Sporn, M. B. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3676–3680
- Chandrasekhar, S. & Harvey, A. K. (1988) *Biochem. Biophys. Res. Commun.* **157**, 1352–1359
- Taylor, D. J., Feldmann, M., Evanson, J. M. & Woolley, D. E. (1989) *Rheumatol. Int.* **9**, 65–70