Rapid Publication

Effect of Platelet-activating Factor and Serum-treated Zymosan on Prostaglandin E_2 Synthesis, Arachidonic Acid Release, and Contraction of Cultured Rat Mesangial Cells

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Abstract. The interaction of inflammatory cells and glomerular prostaglandins (PG) may be important during glomerulonephritis. We therefore examined the influence of platelet-activating factor (PAF), (a mediator of inflammation released from leukocytes) and of phagocytosis of zymosan on arachidonic acid metabolism and on cell contractility in rat glomerular mesangial cells in culture. PAF increased PGE_2 synthesis (determined by radioimmunoassay) within minutes (threshold: 10^{-10} M; maximal effect: 10^{-7} M). Serum-treated zymosan also stimulated PGE_2, but with a slower onset. In cells prelabeled with [^{14}C]arachidonic acid both PAF and serum-treated zymosan released ^{14}C from phospholipids and increased free [^{14}C]arachidonate. The ratio of ^{14}C-release to PGE_2 was, however, different with PAF and serum-treated zymosan, indicating different phospholipid pools. Under phase-contrast microscopy, PAF caused contraction of mesangial cells with a dose-response and time-course parallel to that for PGE_2 synthesis. Serum-treated zymosan caused no contraction. The PAF-induced contraction was enhanced by PG synthesis inhibition and was attenuated by addition of PGE_2, indicating a feedback mechanism. The mesangial contraction by PAF may be important in favoring deposition of immune complexes, while the PGE_2 synthesis stimulated by PAF and by phagocytosis of zymosan may counteract the deleterious effects of PAF during induction of glomerulonephritis.

Introduction

Prostaglandins (PG)[1] may be important in maintaining glomerular function during glomerulonephritis (1). They could be beneficial in a dual manner. First, they attenuate the effect of vasoconstrictor agents such as angiotensin II (1, 2). Such attenuation could also decrease the glomerular deposition of macromolecules and immune complexes, which is enhanced by vasoactive agents, including angiotensin II (3). Second, PGE_2 could inhibit the function and proliferation of inflammatory cells (4). Consistent with this formulation, administration of PGE_1 has been reported to improve immune complex glomerulonephritis (5). During glomerulonephritis, PG synthesis could be influenced by phagocytosis, by deposition of macromolecules in mesangial cells, or by release of mediators of inflammation from leukocytes.

Recently, platelet-activating factor (PAF-acether, [1-0-akyl-2-*O*-acetyl-*SN*-glycero-3-phosphocholine]) has been recognized as a potent mediator of inflammation that is liberated from leukocytes, macrophages, and platelets upon both immunological and nonimmunological stimulation (6). Release of PAF-acether from the isolated perfused rat kidney has also been

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1. *Abbreviations used in this paper:* PAF-acether, platelet-activating factor; PG, prostaglandin; PGE_1 and PGE_2, prostaglandins PGE_1 and PGE_2; STZ, serum-treated zymosan; TLC, thin-layer chromatography.

described (7). PAF-acether causes contraction of smooth muscles in, e.g., isolated ileum (8) as well as vasoconstriction and increased permeability of blood vessels (9). PAF-acether also releases arachidonic acid from phospholipids in platelets (10) and leukocytes (11).

PAF-acether may contribute to glomerulonephritis by increasing capillary permeability and by favoring deposition of immune complexes (12). Phagocytosis of immune complexes by leukocytes in turn increases arachidonic acid metabolism (13, 14) and PAF-acether release (15). Mesangial cells also release lipoxygenase products of arachidonate after phagocytosis of serum-treated zymosan (STZ) (16). We therefore examined the effect of zymosan and PAF-acether on arachidonic acid release, PGE_2 synthesis, and contraction of mesangial cells cultured from rat glomeruli.

Methods

Mesangial cells from rat glomeruli were cultured as reported (17). For the experimental incubation the culture medium was discarded and the flask washed twice with 5 ml of buffer (20 mM Tris-HCl, pH 7.4, 5 mM glucose, 135 mM NaCl, 10 mM KCl, 10 mM Na-acetate, and 3 mM $CaCl_2$) containing 2 mg/ml of fatty acid free bovine serum albumin (BSA). Incubations were in 5 ml of buffer at 30°C (in order to slow down the reaction). Aliquots (0.5 ml) were removed at the times indicated. Radioimmunoassay for PGE_2 (the major PG produced by mesangial cells) (2, 17) was directly determined on the incubation buffer (in duplicate and at least at two different dilutions) (17).

Mesangial cells were prelabeled for 2 h with [^{14}C]arachidonic acid (1.5 μCi/flask), washed twice with medium containing 2 mg/ml BSA, and then recultured in complete medium for 20 h. After two more washes, cells were incubated in 5 ml of buffer at 30°C for 5 min, and aliquots were used for determination of ^{14}C-radioactivity and radioimmunoassay of PGE_2.

Cellular lipids were extracted and developed on silica HL plates (E. Merck, Federal Republic of Germany) in chloroform/methanol/acetic acid/H_2O (15:45:12:3 v/v) (first dimension) and chloroform/methanol/ammonium hydroxide/H_2O (70:30:1:4) (second dimension). Neutral lipids were sequentially developed in chloroform/methanol/H_2O (65:35:5) up to 5 cm and in hexane/diethyleter/formic acid (90:60:4). Radioactivity was determined by counting scraped silica after localization by autoradiography.

Zymosan A (Sigma Chemical Co., St. Louis, MO) was incubated with fresh rat serum for 30 min at 37°C, washed and used immediately. Synthetic PAF-acether and lyso-PAF-acether, gifts from Dr. J. J. Godfroid, University of Paris VII, France, were made up in buffer.

Direct observations of mesangial cells were carried out at room temperature (22°C) under phase-contrast with an Amplival microscope (Zeiss, Veb, Jena, Federal Republic of Germany) equipped with a photographic set. Photographs were taken at 1–5-min intervals.

In an attempt to evaluate the changes in a semiquantitative manner, cell surface area of contractile cells (1–3 cells per serial photograph) was determined with a model 1200 electronic planimeter, (Numonics Corp., Lansdale, PA).

Results

Effects of PAF-acether and zymosan on PGE_2 synthesis. PGE_2 synthesis by mesangial cells increased over a 30-min period (Fig. 1 *A*). PAF-acether (10^{-6} M) rapidly increased PGE_2 (10–15-fold) within 5 min and then leveled off (Fig. 1 *A*). Dose-response experiments with PAF-acether showed stimulation of PGE_2 at concentrations as low as 10^{-10} M, with half maximal and maximal stimulation at 10^{-8} M and 10^{-7} M, respectively (Fig. 1 *B*). Lyso-PAF-acether, lacking the acetyl group in position 2, had no significant effect on PGE_2 synthesis (control: 563±82 pg/culture flask × 20 min; Lyso-PAF-acether 10^{-6} M: 693±54; n = 3). STZ (0.5 mg/ml) stimulated PGE_2 (5–10-fold), progressing up to at least 20 min of incubation (Fig. 1 *A*). Nonactivated zymosan had no effect (Fig. 1 *A*). Dose-response experiments with activated zymosan showed a progressive stimulation of PGE_2 synthesis from controls of 271±52 pg/culture × 20 min, to 637±82 with 0.125 mg/ml of zymosan, to 1,365±118 with 0.25 mg/ml, to 2,036±228 with 0.5 mg/ml, and to 2024±72 with 1 mg/ml of zymosan (two sets of experiments).

Effect of PAF-acether and zymosan on prelabeled cells. The mechanisms of PAF-acether and zymosan-induced PGE_2 synthesis were examined in [^{14}C]arachidonic acid prelabed mesangial cells. PAF-acether decreased ^{14}C-label of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidic acid, and increased [^{14}C]diglyceride and [^{14}C]arachidonic acid (Table I). More of the [^{14}C]arachidonate released was found intracellularly (7,000 cpm) than was found extracellularly (5,000 cpm). PGE_2 increased ~4.5-fold. (Basal PGE_2 values are higher in these experiments, probably because of the prelabeling with arachidonate.) The ratio of ^{14}C to PGE_2 in the buffer decreased from 7.7 cpm/pg PGE_2 in controls to 2.7 cpm/pg after PAF-acether (Table I), indicating enhanced PGE_2 synthesis from nonlabeled arachidonate. Addition of zymosan (0.5 mg/ml, a maximal concentration) also caused release of ^{14}C-label from phosphatidylcholine, phosphatidylinositol, and probably phosphatidylethanolamine (Table I). This resulted in some increases of [^{14}C]diglyceride and [^{14}C]arachidonate (+4,000 cpm) intracellularly and in a major increase of ^{14}C-label (+12,000 cpm) in the incubation buffer. PGE_2 increased twofold. The ratio of ^{14}C to PGE_2 remained constant with zymosan stimulation (12.2 cpm/pg in controls and 13.6 with zymosan), which was consistent with an unchanged transformation of unlabeled and ^{14}C-labeled arachidonate to PGE_2. Nonactivated zymosan (0.5 mg/ml) had no effect on [^{14}C]arachidonate-labeled cells (two sets of experiments).

Microscopical observations. Appearance of mesangial cells did not change during a 90-min control period. PAF-acether (10^{-6} M) contracted the cells after 2–3 min (even at room temperature), progressed up to 25 min, and persisted up to 60 min. Contraction was best observed on cells with large extensions that markedly diminished in diameter (Fig. 2 *A*). When cellular surface area of contracting cells was computed by morphometry,

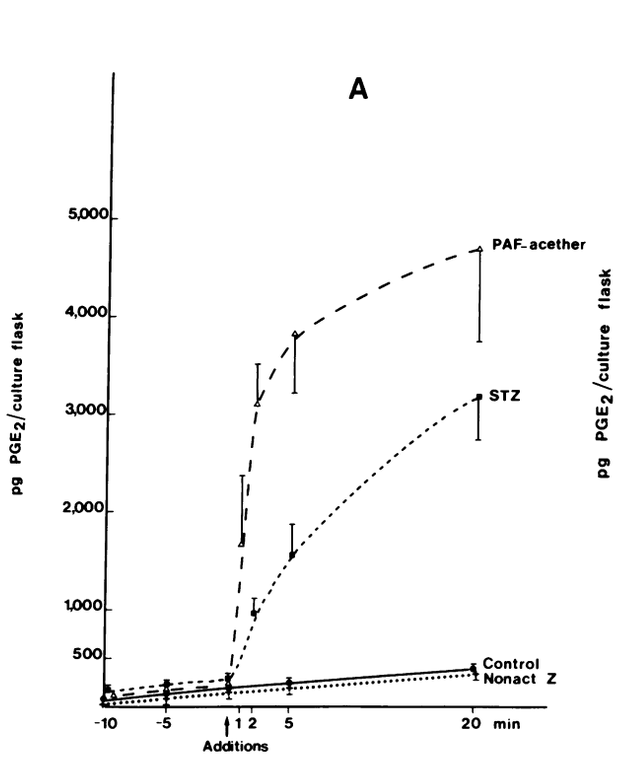

Figure 1. (*A*) Time course of PGE_2 synthesis by cultured mesangial cells under control conditions and after addition of either PAF-acether (10^{-6} M), STZ (0.5 mg/ml), or nonactivated zymosan (0.5 mg/ml). PGE_2 is expressed as picogram per culture flask, corresponding to 4×10^5 cells. Each point represents the mean±SEM of four experiments.

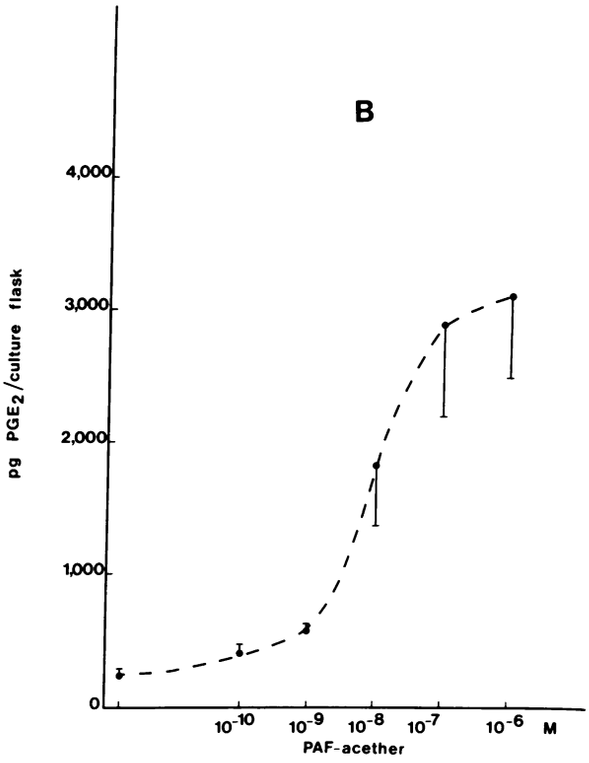

Figure 1. (*B*) Dose-response of PAF-acether on PGE_2 synthesis by mesangial cells (5-min incubations). Each point represents the mean±SEM of three experiments. The increase in PGE_2 at 10^{-10} M PAF-acether is significant ($P < 0.05$) by paired t test.

a decrease to 83.7±2.3% of control was observed 20 min after addition of PAF-acether (10^{-6} M; five sets of experiments). Note that because of marked overlap of cells, only some cells can be fully evaluated by this method. In the presence of the PG-synthesis inhibitor indomethacin (10^{-5} M), PAF-acether (10^{-6} M) resulted in a more rapid and marked decrease in cellular area to 72.7±2.9% of control (three sets of experiments).

Addition of PGE_2 (10^{-6} M) to cells that had contracted in the presence of PAF-acether plus indomethacin resulted in the formation of new cell extensions, consistent with some PGE_2-induced relaxation. (Fig. 2 *B* at 25 min). This was also reflected in the surface area of these cells. After PAF-acether (10^{-9} M) plus inndomethacin, cell surface area had decreased to 79.2±2.3% of control. Within 5 min of further addition of PGE_2 (10^{-6} M), cell area increased to 86.4±2.6% of control (three sets of experiments).

Contraction was discernible at concentrations of PAF-acether as low as 10^{-9} M. PAF-acether (10^{-10} M) alone caused no visible contraction (Fig. 2 *C*, 15 min), but after further addition of indomethacin a notable contraction to 90±2% of control (two sets of experiments) occurred (Fig. 2 *C*, 25 min). Indomethacin alone had no effect. STZ (0.5 mg/ml) caused no contraction.

Discussion

Our results show that PAF-acether and zymosan influence PGE_2 synthesis, phospholipid and arachidonic acid metabolism in cultured mesangial cells. The decrease in ^{14}C-labeled phosphatidylinositol and phosphatidylcholine and the increase in [^{14}C]diglyceride indicates activation of phospholipase C for phosphatidylinositol and phospholipase A_2. Similar observations have been reported with PAF-acether in platelets (10) and leukocytes (11) and with zymosan in macrophages (13, 14). There are, however, several differences in the stimulation by PAF-acether and zymosan. For example, the ratio of ^{14}C to radioimmunoassayable PGE_2 in the buffer decreases from 7.7 cpm/pg PGE_2 in control to 2.7 with PAF-acether, but remains unchanged

Table I. Effect of PAF-Acether and Activated Zymosan on Cultured Mesangial Cells Prelabeled with [^{14}C]Arachidonic Acid

^{14}C In cellular lipids	Control		PAF-acether		Control		Zymosan	
(cpm/4 × 10^5 cells)		*P-value*		*% of control*		*P-value*		*% of control*
Phosphatidyl choline	67,230±2,460	<0.05	59,040±2,740	88	75,080±3,990	<0.025	62,960±5,150	84
Phosphatidyl ethanolamine	37,500±1,020	<0.02	29,340±1,110	78	38,550±4,950	NS	34,260±2,930	89
Phosphatidyl inositol	29,950±3,720	<0.02	23,000±2,650	76	30,090±1,950	<0.025	25,400±1,290	84
Phosphatidyl serine	18,220±2,830	NS	19,680±2,150	108	11,540±2,370	NS	11,720±2,710	101
Phosphatidic acid	5,340±460	<0.01	2,860±320	53	3,860±290	NS	3,160±350	82
Neutral lipids								
Triglycerides	7,890±750	NS	9,280±580	117	4,300±150	NS	4,100±470	95
Diglycerides	1,550±240	<0.05	3,900±710	251	2,330±580	<0.01	4,400±530	189
Monoglycerides	870±100	NS	1,360±150	157	2,540±400	NS	2,860±610	112
Arachidonic acid	4,060±630	<0.05	13,270±2,300	326	4,190±680	<0.01	8,810±430	210
^{14}C In incubation buffer (*cpm*)	7,400±2,760	<0.01	12,090±2,800	163	10,740±680	<0.05	22,740±4,420	212
PGE_2 by radioimmunoassay (pg/4 × 10^5 cells)	960±90	<0.02	4,450±480	463	880±110	<0.02	1,670±180	190
$^{14}C/PGE_2$	7.7		2.7		12.2		13.6	

Cultured mesangial cells were prelabeled with [^{14}C]arachidonic acid and incubated in the absence (controls) or presence of either PAF-acether (10^{-6} M) or STZ (0.5 mg/ml) for 5 min at 30°C. Incubations were terminated by removal of buffer and immediate extraction of cellular lipids. Total ^{14}C-radioactivity and radioimmunoassayable PGE_2 were determined in the incubation buffer. The cellular lipid extract was divided into two halves and used for two-dimensional thin-layer chromatography of phospholipids and thin-layer chromatography of neutral lipids, respectively. For details, see Methods section. Results are mean±SEM (expressed 4 × 10^5 cells) and are from three sets of experiments for PAF-acether and from five sets of experiments for zymosan (with the exception of neutral lipids, which were only determined in three of the five sets of experiments with zymosan). *P*-values refer to comparison by paired *t* test between the values for the respective controls and PAF-acether or zymosan. NS, not significant.

with zymosan (12.2 control, 13.6 zymosan). Thus PAF-acether may stimulate a specific phospholipid-arachidonic acid pool, while zymosan may activate a nonspecific one. This hypothesis is similar to the one proposed by Schwartzman, Lieberman, and Raz (18) for stimulation with angiotensin II in the isolated kidney and by us in mesangial cells (Schlondorff et al., submitted for publication). The stimulation of arachidonic acid release by zymosan increases not only PGE_2 synthesis, but also that of lipoxygenase products, (16) and may be related to phagocytosis of zymosan, similar to observations in macrophages (13, 14). The zymosan-induced production of PGE_2 and 12-hydroxyeicosatetraenoic acid by mesangial cells may play a role in the inflammatory process in the glomerulus. It remains to be determined whether zymosan can also induce PAF-acether production by mesangial cells, as described in leukocytes (15).

PAF-acether, but not zymosan, contracts mesangial cells with a dose-response and time course similar to the stimulation of PGE_2 synthesis. The contraction is enhanced in the presence of PG-inhibition by indomethacin (occurring even at 10^{-10} M of PAF-acether in the presence of indomethacin) and attenuated by readdition of PGE_2. Thus a feedback mechanism may exist, whereby enhanced PGE_2 production antagonizes the contractile response of PAF-acether, which is similar to the observations with vasoactive hormones (1, 2). The mesangial contraction induced by PAF-acether could be important in the induction of glomerulonephritis.

It is known that vasoactive agents such as angiotensin II enhance deposition of macromolecules in the glomerulus (3). Increased vascular permeability, perhaps secondary to vasoconstriction, has been observed with PAF-acether injection into the skin (9). Similarly, it has been proposed that release of PAF-acether from leukocytes contributes to increased vascular permeability, which would favor the deposition of circulating immune complexes in the glomerulus during serum sickness (12). On the other hand, the PGE_2 synthesis induced by PAF-acether may counteract such a mechanism in the glomerulus, both by attenuating the constrictor effect and by inhibiting inflammatory cells (4). Such protective mechanisms of PGE_2 action could

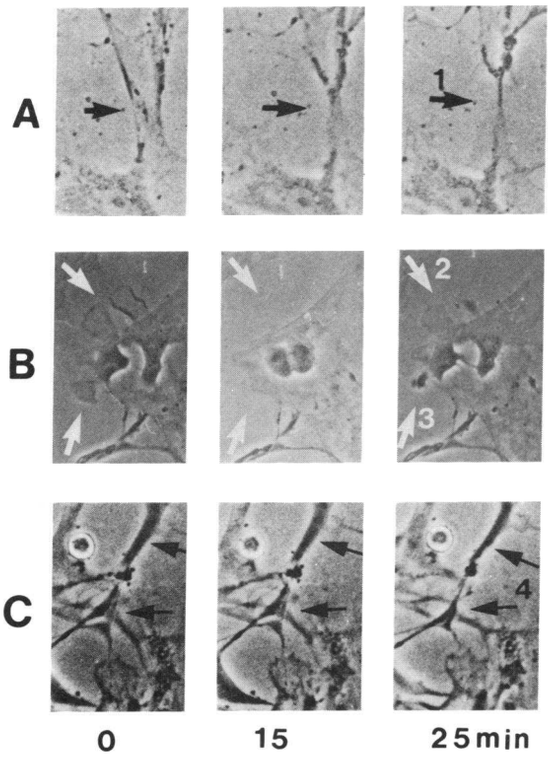

Figure 2. Observation of mesangial cells by phase-contrast microscopy (magnification × 500). Mesangial cells have a stellate appearance with a central nucleus and multiple cytoplasmic extensions frequently containing dark granules. Row *A* shows the effect of PAF-acether (10^{-6} M) with diminution of the diameter of cell extensions (arrow No. *1* showing 80% reduction in diameter) and increased cytoplasmic density. Row *B* shows retraction of cell extensions by PAF-acether (10^{-6} M) in the presence of indomethacin (10^{-5} M) up to 15 min. The picture at 25 min is 5 min after the addition of PGE_2 (10^{-6} M), showing reformation of cell extensions (note arrow No.(s) *2* and *3* on the right). Row *C* shows no appreciable effect of PAF-acether (10^{-10} M) alone (up to 15 min) but notable contraction at 25 min, 5 min after addition of indomethacin (10^{-5} M). Note diminuition of diameters of cell extensions of arrow No. *4*.

also explain the deleterious effect of PG synthesis inhibitors and the beneficial effect of PGE administration on glomerular function during glomerulonephritis (1, 5). The possibility that PAF-acether also increases lipoxygenase products in mesangial cells, as it does in leukocytes (11), remains to be determined.

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