ELSEVIER

European Journal of Pharmacology 434 (2002) 187–196

ejp

www.elsevier.com/locate/ejphar

Participation of prostaglandin E_2 and platelet-activating factor in thapsigargin-induced production of interleukin-6

Gaku Ichinowatari, Masateru Yamada, Hiroshi Yaginuma, Kousei Tsuyuki, Atsuo Tanimoto, Kazuo Ohuchi *

Laboratory of Pathophysiological Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Miyagi 980-8578, Japan

Received 16 August 2001; received in revised form 14 November 2001; accepted 20 November 2001

Abstract

Incubation of rat peritoneal macrophages in the presence of thapsigargin increased production of prostaglandin E_2, intracellular platelet-activating factor (PAF) and interleukin-6. However, no PAF was detected in the conditioned medium. In the presence of SK&F 98625 (diethyl 7-(3,4,5-triphenyl-2-oxo-2,3-dihydroimidazol-1-yl)heptane phosphonate), a CoA-independent transacylase inhibitor, the thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production were suppressed in a concentration-dependent manner. This inhibitor also suppressed the production of prostaglandin E_2 and intracellular PAF. The PAF receptor antagonists such as E6123 ((*S*)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′:4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine) and L-652,731 (2,5-bis(3,4,5-trimethylphenyl)tetrahydrofuran) partially inhibited the thapsigargin-induced increase in the levels of interleukin-6 mRNA and interleukin-6 protein. The SK&F 98625-induced suppression of interleukin-6 mRNA accumulation and interleukin-6 production was partially restored by addition of exogenous prostaglandin E_2. However, exogenous PAF failed to reverse the suppression suggesting that the intracellular PAF does not act in an autocrine mechanism. These findings suggested that the concurrently produced prostaglandin E_2 and intracellular PAF participate in the thapsigargin-induced increase in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages.

Keywords: PAF (platelet-activating factor); Interleukin-6; Prostaglandin E_2; Thapsigargin; SK&F 98625; Macrophage

1. Introduction

CoA-independent transacylase activity has been characterized in various inflammatory cells (Venable et al., 1991; Winkler et al., 1991). The most commonly described aspect of this enzyme is its marked preference for removing arachidonic acid from 1-*O*-alkyl-2-arachidonyl-*sn*-glycero-3-phosphocholine and transferring it into 1-alkyl- and 1-alk-1′-enyl-linked phospholipids in the absence of acyl-CoA (Sugiura et al., 1990; Tessner et al., 1990; Nieto et al., 1991).

Platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a biologically active lipid mediator in inflammatory and allergic reactions (Demopoulos et al., 1979). PAF production by inflammatory cells is thought to proceed via the remodeling pathway (Uemura et al., 1991), which involves the hydrolysis of 1-alkyl- and 1-alk-1′-enyl-linked phospholipids by CoA-independent transacylase and phospholipase A_2 releasing acyl fatty acids, predominantly arachidonic acid, and the subsequent acetylation of the resultant lyso-PAF to PAF by acetyl CoA: lyso-PAF acetyltransferase (Uemura et al., 1991; Venable et al., 1991; Winkler et al., 1992). The released arachidonic acid is then metabolized to prostaglandins and leukotrienes by cyclooxygenase and lipoxygenase, respectively. Therefore, it has been suggested that CoA-independent transacylase is a key enzyme involved in the transacylation of arachidonic acid from phospholipids and in the production of proinflammatory lipid mediators such as PAF, prostaglandins and leukotrienes. We previously reported that, in rat peritoneal macrophages, stimulation by the endomembrane Ca^{2+}-ATPase inhibitor thapsigargin (Thastrup et al., 1987) increased the release of arachidonic acid from membrane phospholipids and the production of both prostaglandin E_2 (Ohuchi et al., 1987, 1988) and intracellular PAF (Watanabe et al., 1992, 1995) within a short incubation period. The levels of PAF in the conditioned medium were less than the

* Corresponding author. Tel.: +81-22-217-6860; fax: +81-22-217-6859.
E-mail address: ohuchi-k@mail.pharm.tohoku.ac.jp (K. Ohuchi).

0014-2999/02/$ - see front matter
PII: S0014-2999(01)01547-3

limits of detection (< 1.0 pmol/ml) (Watanabe et al., 1995). Furthermore, in the presence of the cyclooxygenase inhibitors such as indomethacin, naproxen and ibuprofen, the thapsigargin-induced production of intracellular PAF was further increased to compensate for the decrease in prostaglandin E_2 production (Watanabe et al., 1995; Yamada et al., 1996). Therefore, we suggested that there is a close relationship between the production of both prostaglandin E_2 and intracellular PAF in thapsigargin-stimulated macrophages.

Interleukin-6 is an inflammatory cytokine that is produced by B cells, T cells and macrophages, which contributes to several inflammatory and immune responses (Van Snick, 1990; Bonta and Ben-Efraim, 1993; Bost and Mason, 1995). In inflammatory diseases such as rheumatoid arthritis and multiple myeloma, interleukin-6 is overexpresssed in their tissues (Manicourt et al., 1993; Van Zaanen et al., 1996). The interleukin-6 gene includes sequences similar to the nuclear factor (NF)-κB enhancer (Yoshida et al., 1999), and induction of interleukin-6 production is mediated by the activation of the NF-κB pathway (You et al., 2001). NF-κB plays an important role in the production of interleukin-6 (Shimizu et al., 1990) and tumor necrosis factor-α (Shakhov et al., 1990). We previously reported that thapsigargin-induced production of tumor necrosis factor-α is mediated by concurrently produced intracellular PAF in rat peritoneal macrophages (Yamada et al., 1998). From these reports and our previous findings, we hypothesized that the production of interleukin-6 in stimulated macrophages is up-regulated by the concurrent production of intracellular PAF and prostaglandin E_2. The present study is intended to clarify the roles of intracellular PAF and prostaglandin E_2 in the regulation of interleukin-6 production, using the CoA-independent transacylase inhibitor SK&F 98625, and the PAF receptor antagonists E6123 and L-652,731.

2. Materials and methods

2.1. Preparation of rat peritoneal macrophages

Male Sprague–Dawley rats, specific pathogen-free, and weighing 300–350 g (Charles River Japan, Kanagawa, Japan) were used. The rats were treated in accordance with procedures approved by the Animal Ethics Committee in the Graduate School of Pharmaceutical Sciences, Tohoku University, Japan. Rat peritoneal macrophages were harvested 4 days after an intraperitoneal injection with a solution containing 5% (w/v) of both soluble starch (Wako, Osaka, Japan) and Bacto peptone (Difco Laboratories, Detroit, MI, USA) at a dose of 5 ml per 100 g body weight (Ohuchi et al., 1985).

2.2. Macrophage culture

The peritoneal cells were suspended in Eagle's minimal essential medium (Nissui, Tokyo, Japan) containing 10% (v/v) calf serum (Flow Laboratories, North Ryde, NSW, Australia), penicillin G potassium (18 μg/ml) and streptomycin sulfate (50 μg/ml) (Meiji Seika, Tokyo, Japan) at a density of 1.5×10^6 cells per ml of the medium. The cell suspension was poured into plastic tissue culture dishes or plates (Corning Costar, Cambridge, MA, USA) before being incubated for 2 h at 37 °C. The cells were then washed three times with medium to remove non-adherent cells (Ohuchi et al., 1985). The adherent cells were incubated for 20 h at 37 °C in 1 ml per 1.5×10^6 cells of medium containing 10% (v/v) calf serum, and after three further washes with medium containing no calf serum, they were ready for use.

2.3. Preparation of diisopropylfluorophosphate-treated calf serum

Calf serum was treated with 10 mM diisopropylfluorophosphate (DFP, Wako) for 1 h at 37 °C to inactivate the PAF acetylhydrolase (Satoh et al., 1993). The remaining DFP was removed by dialysis against 20 volumes of phosphate-buffered saline (PBS). The dialysis buffer was changed three times at 12-h intervals. To confirm that the DFP-treated calf serum showed no PAF acetylhydrolase activity, the following experiment was performed; 100 μl of the DFP-treated calf serum was incubated for 15 min at 37 °C with 150 μl of 50 mM Tris–HCl buffer (pH 7.4) containing 0.1 kBq of [^{3}H]PAF (1-*O*-alkyl-2-[^{3}H]acetyl-*sn*-glycero-3-phosphocholine, specific radioactivity 370 MBq/mol, DuPont NEN Research Products) and 5 mM EDTA. After the incubation, 2.5 ml of methanol/chloroform (1:4, v/v) and 0.25 ml of water were added, and the radioactivity in the upper phase was measured to determine the amount of [^{3}H]acetate liberated (Hattori et al., 1995). The DFP-treated calf serum showed no PAF acetylhydrolase activity with activity the same as that of heat-treated (98 °C, 5 min) calf serum.

2.4. Incubation of macrophages with drugs

After three washes with medium containing no calf serum, the cells were incubated for the periods indicated at 37 °C in 1 ml per 1.5×10^6 cells of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of various drugs. The drugs used were the endomembrane Ca^{2+}-ATPase inhibitor thapsigargin (LC Services, Woburn, MA, USA), the cyclooxygenase inhibitor indomethacin, prostaglandin E_2, PAF (a mixture of C_{16} and C_{18} forms, 1:1, w/w) (Sigma, St Louis, MO, USA), the CoA-independent transacylase inhibitor SK&F 98625 (diethyl 7-(3,4,5-triphenyl-2-oxo-2,3-dihydroimidazol-1-yl)heptane phosphonate) (Chilton et al., 1995), the PAF receptor antagonists E6123 ((*S*)-(+)-6-(2-chlorophenyl)-3-cyclopropanecarbonyl-8,11-dimethyl-2,3,4,5-tetrahydro-8*H*-pyrido[4′,3′ : 4,5]thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine) (Tsunoda et al., 1990) and L-652,731 (2,5-bis(3,4,5-trimethoxyphe-

nyltetrahydrofuran)) (Hwang et al., 1985). All drugs were dissolved in dimethylsulfoxide. An aliquot of each solution was added to medium, and the final concentration of the vehicle in medium was adjusted to 0.1% (v/v). The control medium contained the same amount of the vehicle.

2.5. Viability assay

The viability of the cells was examined in each set of experiments by a procedure using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma) (Mosmann, 1983; Tada et al., 1986), which is based on the ability of mitochondrial succinate dehydrogenase to cleave MTT to the blue compound formazan. After the cells were incubated for the periods indicated in 10 ml of medium containing the various drugs, 1 ml of MTT solution in phosphate-buffered saline (5 mg/ml, pH 7.4) was added to each dish, and the cells were further incubated for 4 h at 37 °C. Next, 2.5 ml of 0.04 N HCl solution in isopropanol was added, and the cells were sonicated using a Handy Sonic Disrupter (UR-20P, Tomy, Tokyo, Japan) at 10% maximum power for 3 s. The resultant colored product was read on a Microplate Reader (Bio-Rad, Richmond, CA, USA) at 570 nm. None of the drugs given at concentrations described in this paper had any significant effect on cell viability.

2.6. Measurement of interleukin-6

The conditioned medium was centrifuged at $1500 \times g$ and 4 °C for 5 min. Interleukin-6 concentrations in the supernatant fraction were immunoassayed using a commercially available kit (Cytoscreen™ Rat Interleukin-6 ELISA Kit, Biosource, Camarillo, CA, USA). The procedure for the immunoassay is described in the instruction manual accompanying the kit.

2.7. Semi-quantitation of interleukin-6 mRNA by reverse transcription-polymerase chain reaction (RT-PCR)

Peritoneal macrophages were incubated for the periods indicated at 37 °C in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of various drugs. After the incubation, total RNA was prepared from each sample by acid guanidium-phenol-chloroform extraction (Chomczynski and Sacchi, 1987), and the yield of RNA was determined by spectrophotometry. A total of 1 μg of RNA from each sample was reverse transcribed at 37 °C for 1 h in 20 μl of buffer (50 mM Tris–HCl, pH 8.3, 75 mM KCl and 3 mM $MgCl_2$) containing 5 μM of random hexamer oligonucleotides (Gibco, Gaithersburg, MD, USA), 200 U of the reverse transcriptase from moloney murine leukemia virus (Gibco), 0.5 mM deoxyribonucleotide triphosphates (dNTP, Pharmacia Biotech, Uppsala, Sweden) and 10 mM dithiothreitol. The PCR primers for interleukin-6 were designed according to Nadeau et al. (1995); (forward) 5′ -CA AGAGACTTCCAGCCAGTTGC-3′ and (reverse) 5′ -TTG CCGAGTAGACCTCATAGTGACC-3′ , which amplify a 614 base pair interleukin-6 fragment. PCR was performed for 27 cycles in 50 μl of PCR buffer (2.5 mM Tris–HCl, pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$) containing 5 μM of the reverse transcribed RNA solution, 0.25 μM of each primer, 170 μM dNTP and 1.25 U Taq polymerase (Takara Shuzo, Shiga, Japan) using a thermal cycler (GeneAmp™ PCR System 2400, Perkin Elmer Cetus, Norwalk, CT, USA). Each cycle consisted of 30 s denaturation at 94 °C, 1 min annealing at 57 °C, and 2 min extension at 72 °C.

The rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (a housekeeping gene) was used as the internal standard gene. Thus as the internal standard gene was also amplified, relative levels of interleukin-6 mRNA could

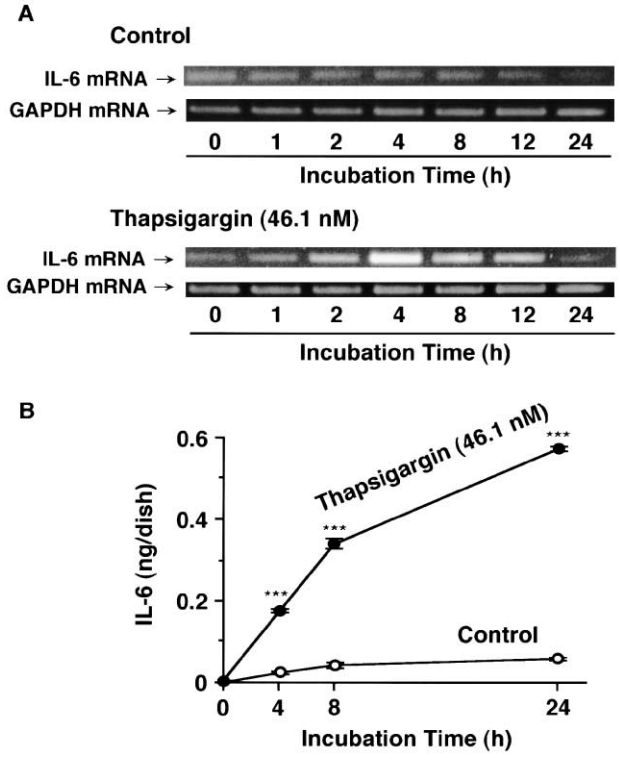

Fig. 1. Time course of thapsigargin-induced accumulation of interleukin-6 mRNA and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 20 h in 10 ml of medium containing 10% (v/v) calf serum. After three washes with medium, the cells were incubated at 37 °C for the periods indicated in 10 ml of medium containing 10% (v/v) DFP-treated calf serum with or without thapsigargin (30 ng/ml; 46.1 nM). Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 20 h in 1 ml of medium containing 10% (v/v) calf serum. After three washes with medium, the cells were incubated at 37 °C for the periods indicated in 1 ml of medium containing 10% (v/v) DFP-treated calf serum with (●) or without (○) thapsigargin (30 ng/ml; 46.1 nM). IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P < 0.001$ vs. corresponding control.

Table 1
Effects of various concentrations of thapsigargin on interleukin-6 production by rat peritoneal macrophages

Treatment	Concentration (nM)	IL-6 (ng/dish)
Thapsigargin	0	0.05 ± 0.01
	4.61	0.11 ± 0.01[a]
	46.1	0.34 ± 0.02[b]
	461.1	0.41 ± 0.03[b]

Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum and the indicated concentrations of thapsigargin. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means with S.E.M. from four samples.

[a] $P < 0.05$ vs. control.

[b] $P < 0.001$ vs. control.

be quantified. PCR primers for rat GAPDH were described by Robbins and McKinney (1992). The primers used were (forward) 5′ -TGATGACAAGAAGGTGGTGAAG-3′ , and (reverse) 5′ -TCCTTGGAGGCCATGTAGGCCAT-3′ , which amplify a 240 base pair GAPDH fragment. PCR was performed for 27 cycles, each cycle consisted of 30 s denaturation at 94 °C, 1 min annealing at 57 °C, and 2 min extension at 72 °C. Other conditions were the same as for interleukin-6 mRNA. After PCR, 10 μl of the reaction mixture was loaded onto a 1.5% agarose mini-gel, and the PCR products were visualized by ethidium bromide staining after electrophoresis. The levels of mRNA for interleukin-6 and GAPDH were quantified by scanning densitometry, and the ratio of the interleukin-6 mRNA density vs. the GAPDH mRNA density at each point was calculated.

2.8. Measurement of prostaglandin E_2

The conditioned medium was centrifuged at $1500 \times g$ and 4 °C for 5 min. Prostaglandin E_2 concentrations in the supernatant fraction were measured by a radioimmunoassay (Ohuchi et al., 1985). Prostaglandin E_2 antiserum was purchased from PerSeptive Diagnostics, Cambridge, MA, USA.

2.9. Measurement of cell-associated PAF

After partial purification of PAF from the total lipid fraction of the cells or from the conditioned medium using an immunoaffinity mini-column (Watanabe et al., 1992), PAF contents were measured using a commercially available kit (Platelet Activating Factor [^{125}I]RIA Kit, DuPont NEN Reserch Products) as described previously (Watanabe et al., 1992).

2.10. Statistical significance

Results were analyzed for statistical significance by Dunnett's test for multiple comparison and Student's *t*-test for unpaired observations.

3. Results

3.1. Effects of thapsigargin on the interleukin-6 mRNA level and interleukin-6 production

Time-course changes in the accumulation of interleukin-6 mRNA and production of interleukin-6 by rat peritoneal macrophages in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) were examined. In the presence of thapsigargin, the interleukin-6 mRNA level was increased in a time dependent manner, reaching a maximum at 4 h, and declined thereafter until 24 h. Levels of GAPDH mRNA did not change during the incubation period (Fig. 1A). In the absence of thapsigargin, no prominent change in the interleukin-6 mRNA level was observed before 24 h (Fig. 1A). When the cells were examined after a 4-h incubation with thapsigargin, the interleukin-6 mRNA level was found to increase in a concentration-dependent manner at 4.61–461.1 nM (data not shown). Interleukin-6 concentrations in the conditioned medium of thapsigargin-stimulated mac-

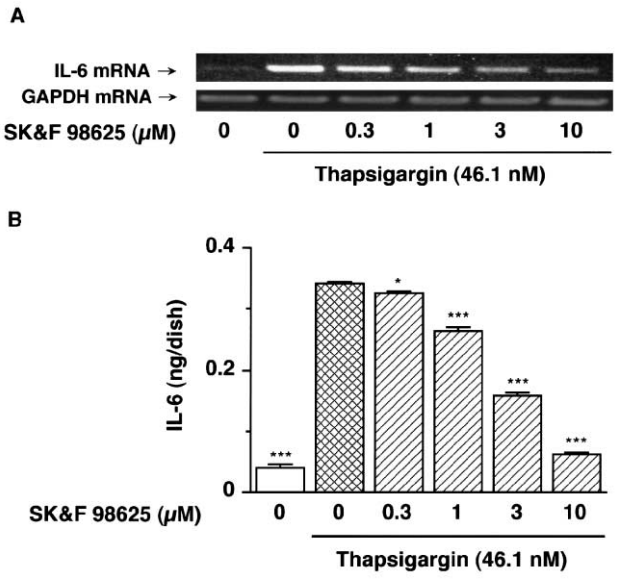

Fig. 2. Effects of various concentrations of SK&F 98625 on thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of SK&F 98625. Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of SK&F 98625. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: * $P < 0.05$, *** $P < 0.001$ vs. thapsigargin control.

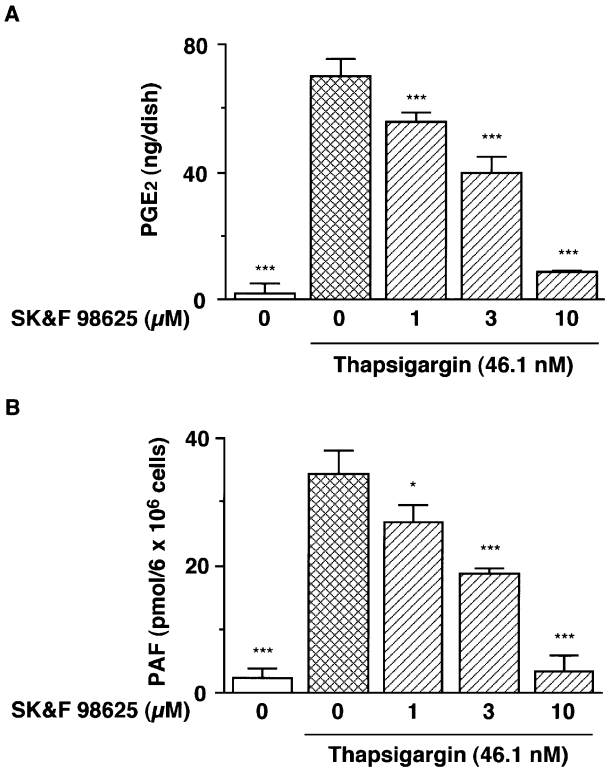

Fig. 3. Effects of SK&F 98625 on thapsigargin-induced production of prostaglandin E_2 and cell-associated PAF by rat peritoneal macrophages. Rat peritoneal macrophages (6.0×10^6 cells) were incubated at 37 °C for 10 min in 4 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of SK&F 98625. The levels of prostaglandin E_2 (PGE_2) in the conditioned medium (A) and cell-associated PAF (B) were determined as described in Sections 2.8 and 2.9, respectively. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P < 0.001$ vs. thapsigargin control.

rophages were increased time-dependently with significant increases detected at 4, 8 and 24 h (Fig. 1B). The amount of interleukin-6 in the conditioned medium at 8 h were increased by thapsigargin in a concentration-dependent manner at 4.61–461.1 nM (Table 1).

3.2. Effects of SK&F 98625, a CoA-independent transacylase inhibitor, on the thapsigargin-induced increase in the interleukin-6 mRNA level and interleukin-6 production

Treatment with SK&F 98625 (0.3–10 μM) inhibited both the thapsigargin (46.1 nM)-induced increase in the interleukin-6 mRNA level at 4 h (Fig. 2A) and the interleukin-6 production at 8 h in a concentration-dependent manner (Fig. 2B). These findings suggested that CoA-independent transacylase is an enzyme involved in the increased interleukin-6 mRNA level and interleukin-6 production in thapsigargin-stimulated rat peritoneal macrophages.

3.3. Effects of SK&F 98625 on thapsigargin-induced production of prostaglandin E_2 and cell-associated PAF

After a 10-min incubation with SK&F 98625 (0.3–10 μM), the thapsigargin (46.1 nM)-induced production of prostaglandin E_2 and cell-associated PAF was suppressed in a concentration-dependent manner (Fig. 3). The amounts of PAF in the conditioned medium were less than the detectable limit (< 1.0 pmol/ml). These findings suggested that the concurrently produced prostaglandin E_2 and cell-associated PAF participate in the thapsigargin-induced up-regulation of the interleukin-6 mRNA level and interleukin-6 production.

3.4. Effects of E6123 and L-652,731, PAF receptor antagonists, on the thapsigargin-induced increase in the interleukin-6 mRNA level and interleukin-6 production

To clarify the role of the concurrently produced cell-associated PAF in the thapsigargin-induced increase in the

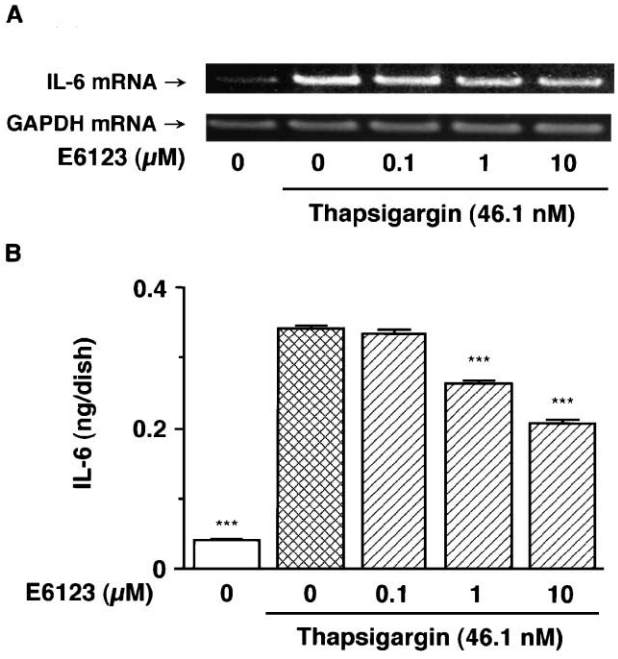

Fig. 4. Effects of various concentrations of E6123 on thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of E6123. Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of E6123. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P < 0.001$ vs. thapsigargin control.

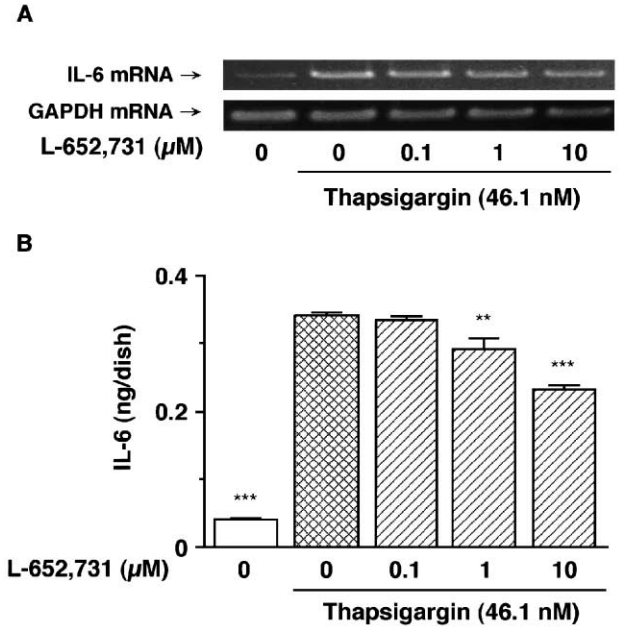

Fig. 5. Effects of various concentrations of L-652,731 on thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of L-652,731. Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of L-652,731. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: ** $P<0.01$, *** $P<0.001$ vs. thapsigargin control.

interleukin-6 mRNA level and interleukin-6 production, the effects of the PAF receptor antagonists E6123 and L-652,731 were examined. E6123 at 1 and 10 μM partially inhibited the thapsigargin (46.1 nM)-induced increase in the interleukin-6 mRNA level at 4 h (Fig. 4A) and interleukin-6 production at 8 h (Fig. 4B). L-652,731 at 1 and 10 μM also partially inhibited the increase in the interleukin-6 mRNA level at 4 h and interleukin-6 production at 8 h (Fig. 5). These findings suggested that the concurrently produced cell-associated PAF is partially involved in thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production.

3.5. Effects of exogenously added prostaglandin E_2 on SK&F 98625-induced suppression of the interleukin-6 mRNA level and interleukin-6 production in thapsigargin-stimulated macrophages

To clarify the role of the concurrently produced prostaglandin E_2 in thapsigargin-induced increases in interleukin-6 mRNA level and interleukin-6 production, the effects of exogenously added prostaglandin E_2 on SK&F 98625-induced suppression of the interleukin-6 mRNA level and interleukin-6 production in thapsigargin-stimulated macrophages were examined. Treatment with 10 μM of SK&F 98625 reduced the thapsigargin (46.1 nM)-induced increase in prostaglandin E_2 production to the control level (Fig. 3B). Exogenously added prostaglandin E_2 (1–100 nM) reversed the SK&F 98625-induced suppression of the interleukin-6 mRNA level at 4 h (Fig. 6A), and interleukin-6 production at 8 h in a concentration-dependent manner (Fig. 6B). These findings suggested that the concurrently produced prostaglandin E_2 up-regulates the interleukin-6 mRNA level and interleukin-6 production in hapsigargin-stimulated rat peritoneal macrophages.

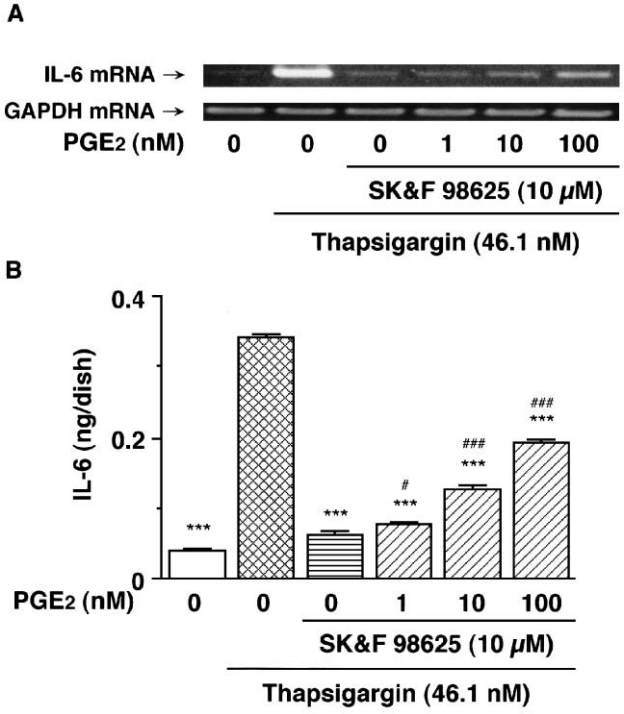

Fig. 6. Effects of exogenously added prostaglandin E_2 on SK&F 98625-induced suppression of thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM), SK&F 98625 (10 μM) and the indicated concentrations of prostaglandin E_2 (PGE_2). Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM), SK&F 98625 (10 μM) and the indicated concentrations of PGE_2. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P<0.001$ vs. thapsigargin control and $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ vs. thapsigargin plus SK&F 98625.

3.6. Effects of exogenously added PAF on SK&F 98625-induced suppression of the interleukin-6 mRNA level and interleukin-6 production in thapsigargin-stimulated macrophages

Effects of exogenously added PAF on the SK&F 98625-induced suppression of the interleukin-6 mRNA level and interleukin-6 production in thapsigargin-stimulated macrophages were examined. Exogenously added PAF (1-100 nM) did not reverse the SK&F 98625-induced suppression of the interleukin-6 mRNA level at 4 h (Fig. 6A), and interleukin-6 production at 8 h (Fig. 6B). Furthermore, exogenously added PAF (1–100 nM) by itself did not induce interleukin-6 production (data not shown). These findings suggested that cell-associated PAF produced by thapsigargin treatment does not act to produce interleukin-6 by an autocrine mechanism. Therefore, the cell-associated PAF might play a significant role as an intracellular signaling molecule in interleukin-6 production.

3.7. Effects of indomethacin on the thapsigargin-induced production of prostaglandin E_2, cell-associated PAF and interleukin-6, and increase in the interleukin-6 mRNA level

After a 10-min incubation with indomethacin (0.01–1 μM), the thapsigargin (46.1 nM)-induced production of prostaglandin E_2 was inhibited, while the thapsigargin-induced production of cell-associated PAF was further potentiated in a concentration-dependent manner (Table 2). The amounts of PAF in the conditioned medium were less than the detectable limit (<1.0 pmol/ml). Indomethacin (0.01–1 μM) did not affect the thapsigargin-induced production of interleukin-6 or increase in the interleukin-6 mRNA level (Fig. 8).

Table 2
Effects of indomethacin on thapsigargin-induced production of prostaglandin E_2 and cell-associated PAF by rat peritoneal macrophages

Treatment	PGE_2 (ng/dish)	PAF (pmol/6×10^6 cells)
None	2.78 ± 0.05[a]	2.22 ± 0.89[a]
Thapsigargin (46.1 nM)	71.11 ± 4.44	30.00 ± 2.22
+ Indomethacin (0.01 μM)	61.67 ± 6.67[a]	35.56 ± 2.78[b]
+ Indomethacin (0.1 μM)	40.78 ± 3.33[a]	45.56 ± 3.00[c]
+ Indomethacin (1 μM)	8.89 ± 1.44[b]	61.11 ± 2.33[a]

Rat peritoneal macrophages (6.0×10^6 cells) were incubated at 37 °C for 10 min in 4 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of indomethacin. The levels of prostaglandin E_2 (PGE_2) in the conditioned medium and cell-associated PAF were determined as described in Sections 2.8 and 2.9, respectively. Values are the means from four samples with S.E.M. shown by vertical bars.

[a] $P<0.001$ vs. thapsigargin.
[b] $P<0.05$ vs. thapsigargin.
[c] $P<0.01$ vs. thapsigargin.

4. Discussion

In rat peritoneal macrophages, we reported that treatment with thapsigargin increases the release of arachidonic acid (Ohuchi et al., 1988), production of prostaglandin E_2 (Ohuchi et al., 1987), cell-associated PAF (Watanabe et al., 1995) and secretion of tumor necrosis factor-α (Yamada et al., 1998). It has also been reported that thapsigargin treatment increases the production of interleukin-6 in mouse peritonreal macrophages (Bost and Mason, 1995). Therefore, in this study, we pharmacologically analyzed the role of the concurrently produced prostaglandin E_2 and cell-associated PAF in thapsigargin-stimulated production of interleukin-6. It was demonstrated that treatment with SK&F 98625, a CoA-independent transacylase inhibitor, inhibited thapsigargin-induced production of interleukin-6 (Fig. 2), prostaglandin E_2 and cell-associated PAF in a concentration-dependent manner (Fig. 3). Winkler et al. (1995) also reported that SK&F 98625 inhibits production of prostaglandin E_2 and PAF in A23187-stimulated human neutrophils. In addition, PAF receptor antagonists such as E6123 (an analog of triazolobenzodiazepine) and L-652,731 (an analog of tetrahydrofuran) partially inhibited the thapsigargin-induced interleukin-6 production (Figs. 4 and 5). However, under the suppression of intracellular PAF production by SK&F 98625, exogenously added PAF could not reverse the SK&F 98625-induced suppression of interleukin-6 production (Fig. 7). In contrast, exogenously added prostaglandin E_2 (1–100 nM) partially reversed the SK&F 98625-induced suppression of interleukin-6 production in thapsigargin-stimulated macrophages (Fig. 6). Enhancement of interleukin-6 production by prostaglandin E_2 has also been reported in macrophages and fibroblasts (Agro et al., 1996; Hinson et al., 1996). In this study, we showed that the CoA-independent transacylase inhibitor SK&F 98625 inhibited the thapsigargin-induced increase in the production of interleukin-6 by lowering the level of interleukin-6 mRNA (Fig. 2). These findings suggested that thapsigargin-induced production of interleukin-6 is up-regulated by concurrently produced cell-associated PAF and prostaglandin E_2. However, we previously reported that thapsigargin-induced production of tumor necrosis factor-α is up-regulated by concurrently produced cell-associated PAF and down-regulated by prostaglandin E_2 (Yamada et al., 1999). Therefore, the mechanism of regulation for the thapsigargin-induced interleukin-6 production is different from that of the thapsigargin-induced tumor necrosis factor-α production.

As shown in Figs. 4 and 5, the PAF receptor antagonists partially inhibited the thapsigargin-induced interleukin-6 production. However, the concentrations of these antagonists required for the inhibition of interleukin-6 production (1–10 μM) were higher than those required for the inhibition against exogenous PAF (Watanabe et al., 1994). These findings suggested the possibility that the target of these PAF receptor antagonists for the inhibition of interleukin-6 production is not the PAF receptor on the cell surface.

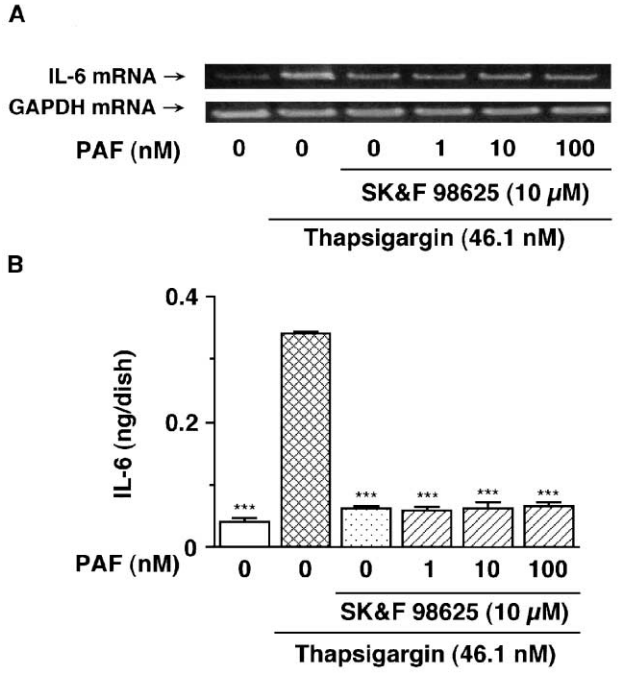

Fig. 7. Effects of exogenously added PAF on SK&F 98625-induced suppression of thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM), SK&F 98625 (10 μM) and the indicated concentrations of PAF. Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM), SK&F 98625 (10 μM) and the indicated concentrations of PAF. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P < 0.001$ vs. thapsigargin control.

As to the role of cell-associated PAF, Stewart and Phillips (1989) speculated that PAF acts as an intracellular messenger for eicosanoid production in guinea-pig peritoneal macrophages. Müller and Nigam (1992) also suggested that PAF produced in human neutrophils reacts with intracellular PAF binding sites to activate phospholipase A_2. In addition, distinct PAF binding sites in plasma membranes and in microsomal membranes were identified in rat cerebral cortex (Marcheselli et al., 1990). Therefore, in thapsigargin-stimulated macrophages, the cell-associated PAF might bind to intracellular binding sites and induce signal transduction which stimulates production of interleukin-6. In this respect, we are currently attempting to clarify the action mechanism of the cell-associated PAF.

As to the action of exogenously added PAF, it has been reported that the binding of exogenous PAF to its cell surface receptor stimulates gene expression of c-*fos* and c-*Jun* in rabbit corneal epithelial cells (Bazan et al., 1993), and induces activation of nuclear factor κB (NF-κB) in stably transfected Chinese hamster ovary cells expressing the PAF receptor (Kravchenko et al., 1995). The interleukin-6 gene includes sequences similar to the NF-κB enhancer (Yoshida et al., 1999). Therefore, it is likely that exogenous PAF induces gene expression of interleukin-6. However, in our present study, exogenously added PAF to the rat peritoneal macrophage cultures in the absence of thapsigargin (46.1 nM) did not induce interleukin-6 production even at concentrations up to 100 nM (data not shown). Furthermore, the addition of PAF did not reverse the SK&F 98625-induced suppression of interleukin-6 production (Fig. 7). In this study, the macrophages were incubated in medium containing DFP-treated serum lacking PAF acetylhydrolase activity, thus it is unlikely that exogenously added PAF was metabolized to lyso-PAF, a biologically inactive form. Therefore, our findings strongly suggested that concurrently produced cell-associated PAF acts as an intracellular signaling molecule for the induction of interleukin-6 production probably through the activation of NF-κB. It is possible that not only the NF-κB activation by cell-associated PAF, but also the NF-IL6 (CCAAT/enhancer-binding protein; C/EBPβ) activation

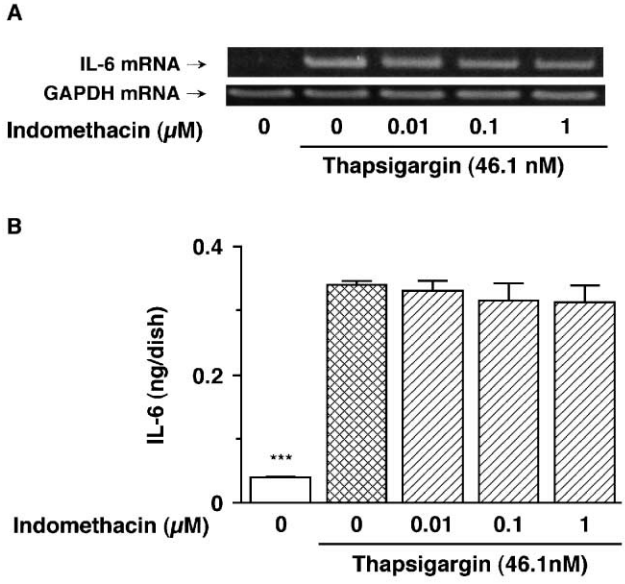

Fig. 8. Effects of various concentrations of indomethacin on thapsigargin-induced increases in the interleukin-6 mRNA level and interleukin-6 production by rat peritoneal macrophages. (A) Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37 °C for 4 h in 10 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of indomethacin. Total RNA was extracted and RT-PCR for interleukin-6 (IL-6) mRNA and GAPDH mRNA was performed. The results shown are representative of three separate experiments. (B) Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37 °C for 8 h in 1 ml of medium containing 10% (v/v) DFP-treated calf serum in the presence or absence of thapsigargin (30 ng/ml, 46.1 nM) and the indicated concentrations of indomethacin. IL-6 concentrations in the conditioned medium were determined by ELISA. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: *** $P < 0.001$ vs. thapsigargin control.

by prostaglandin E_2 participates in the thapsigargin-induced interleukin-6 production, because the interleukin-6 promoter gene possesses the NF-IL6 binding site (Shimizu et al., 1990; Galien et al., 1996; Akira, 1997). It is also possible that the concurrently produced tumor necrosis factor-α up-regulates interleukin-6 production. However, Kiehntopf et al. (1995) reported that NF-IL6 activation is required for tumor necrosis factor-α-inducible expression of the granulocyte colony-stimulating factor (G-CSF), but not the granulocyte/marophage CSF (GM-CSF) or interleukin-6 gene in human fibroblasts.

As shown in Fig. 8, the cyclooxygenase inhibitor indomethacin had no significant effect on thapsigargin-induced interleukin-6 production. We previously reported that the cyclooxygenase inhibitors suppress prostaglandin E_2 production but enhance cell-associated PAF production (Watanabe et al., 1995) as shown in Table 2. Therefore, the lack of effect on thapsigargin-induced interleukin-6 production by indomethacin might be due to the fact that although indomethacin potentiates the production of cell-associated PAF which leads to an up-regulation of interleukin-6 production, this effect is offset by the decreased up-regulation resulting from the reduced prostaglandin E_2 production.

In conclusion, our findings suggest that concurrently produced cell-associated PAF and prostaglandin E_2 up-regulate thapsigargin-induced interleukin-6 production. Cell-associated PAF does not act by an autocrine mechanism, but may act as an intracellular signaling molecule for interleukin-6 production. The PAF receptor antagonists might penetrate into the cells, thus compete with concurrently produced cell-associated PAF to inhibit thapsigargin-induced interleukin-6 production. Further study is necessary to clarify the action mechanism of cell-associated PAF.

Acknowledgements

The present study was supported in part by the Sasakawa Research Grant from the Japan Science Society (to G.I.), and by a Grant-in-Aid for Scientific Research (11470481 to K.O.) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Agro, A., Langdon, C., Smith, F., Richards, C.D., 1996. Prostaglandin E_2 enhances interleukin 8 (IL-8) and IL-6 but inhibits GMCSF production by IL-1 stimulated human synovial fibloblasts in vitro. J. Rheumatol. 23, 862–868.

Akira, S., 1997. IL-6-regulated transcription factors. Int. J. Biochem. Cell Biol. 29, 1401–1418.

Bazan, H.E.P., Tao, Y., Bazan, N.G., 1993. Platelet-activating factor induces collagenase expression in corneal epithelial cells. Proc. Natl. Acad. Sci. U.S.A. 90, 8678–8682.

Bonta, I.L., Ben-Efraim, S., 1993. Involvement of inflammatory mediators in macrophage antitumor activity. J. Leukocyte Biol. 54, 613–626.

Bost, K.L., Mason, M.J., 1995. Thapsigargin and cyclopiazonic acid initiate rapid and dramatic increases of IL-6 mRNA expression and IL-6 secretion in murine peritoneal macrophages. J. Immunol. 155, 285–296.

Chilton, F.H., Fonteh, A.N., Sung, C.M., Hickey, D.M.B., Torphy, T.J., Mayer, R.J., Marshall, L.A., Heravi, J.D., Winkler, J.D., 1995. Inhibitors of CoA-independent transacylase block the movement of arachidonate into 1-ether-linked phospholipids of human neutrophils. Biochemistry 34, 5403–5410.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Demopoulos, C.A., Pinckard, R.N., Hanahan, D.J., 1979. Platelet-activating factor. Evidence for 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). J. Biol. Chem. 254, 9355–9358.

Galien, R., Evans, H.F., Garcia, T., 1996. Involvement of CCAAT/enhancer-binding protein and nuclear factor-kappa B binding sites in interleukin-6 promoter inhibition by estrogen. Mol. Endocrinol. 10, 713–722.

Hattori, K., Hattori, M., Adachi, H., Tsujimoto, M., Arai, H., Inoue, K., 1995. Purification and characterization of platelet-activating factor acetylhydrolase II from bovine liver cytosol. J. Biol. Chem. 270, 22308–22313.

Hinson, R.M., Williams, J.A., Shacter, E., 1996. Elevated interleukin 6 is induced by prostaglandin E_2 in a murine model of inflammation: possible role of cyclooxygenase-2. Proc. Natl. Acad. Sci. U.S.A. 93, 4885–4890.

Hwang, S.B., Lam, M.H., Biftu, T., Beattie, T.R., Shen, T.Y., 1985. *trans*-2,5-Bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran. An orally active specific and competitive receptor antagonist of platelet activating factor. J. Biol. Chem. 260, 15639–15645.

Kiehntopf, M., Herrmann, F., Brach, M.A., 1995. Functional NF-IL6/CCAAT enhancer-binding protein is required for tumor necrosis factor alpha-inducible expression of the granulocyte colony-stimulating factor (CSF), but not the granulocyte/macrophage CSF or interleukin 6 gene in human fibloblasts. J. Exp. Med. 181, 793–798.

Kravchenko, V.V., Pan, Z., Han, J., Herbert, J.M., Ulevitch, R.J., Ye, R.D., 1995. Platelet-activating factor induces NF-κB activation through a G protein-coupled pathway. J. Biol. Chem. 270, 14928–14934.

Manicourt, D.H., Triki, R., Fukuda, K., Devogelaer, J.P., Nagant de Deuxchaisnes, C., Thonar, E.J., 1993. Levels of circulating tumor necrosis factor α and interleukin-6 in patients with rheumatoid arthritis. Relationship to serum levels of hyaluronan and antigenic keratan sulfate. Arthritis Rheum. 36, 490–499.

Marcheselli, V.L., Rossowska, M.J., Domingo, M.T., Braquet, P., Bazan, N.G., 1990. Distinct platelet-activating factor binding sites in synaptic endings and in intracellular membranes of rat cerebral cortex. J. Biol. Chem. 265, 9140–9145.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Müller, S., Nigam, S., 1992. Enhancement by staurosporine of platelet-activating factor formation in *N*-formyl peptide-challenged human neutrophils is mediated by intracellular platelet-activating factor binding sites. Biochem. Biophys. Res. Commun. 189, 771–776.

Nadeau, K.C., Azuma, H., Tilney, N.L., 1995. Sequential cytokine dynamics in chronic rejection of rat renal allografts: roles for cytokines RANTES and MCP-1. Proc. Natl. Acad. Sci. U.S.A. 92, 8729–8733.

Nieto, M.L., Venable, M.E., Bauldry, S.A., Greene, D.G., Kennedy, M., Bass, D.A., Wykle, R.L., 1991. Evidence that hydrolysis of ethanolamine plasmalogens triggers synthesis of platelet-activating factor via a transacylation reaction. J. Biol. Chem. 266, 18699–18706.

Ohuchi, K., Watanabe, M., Yoshizawa, K., Tsurufuji, S., Fujiki, H., Suganuma, M., Sugimura, T., Levine, L., 1985. Stimulation of prostaglandin E_2 production by 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-type and non-TPA-type tumor promoters in macrophages and its inhibition by cycloheximide. Biochim. Biophys. Acta 834, 42–47.

Ohuchi, K., Sugawara, T., Watanabe, M., Hirasawa, N., Tsurufuji, S., Fujiki, H., Sugimura, T., Christensen, S.B., 1987. Stimulation of arach-

idonic acid metabolism in rat peritoneal macrophages by thapsigargin, a non-(12-*O*-tetradecanoylphorbol 13-acetate) (TPA)-type tumor promoter. J. Cancer Res. Clin. Oncol. 113, 319–324.

Ohuchi, K., Sugawara, T., Watanabe, M., Hirasawa, N., Tsurufuji, S., Fujiki, H., Christensen, S.B., Sugimura, T., 1988. Analysis of the stimulative effect of thapsigargin, a non-TPA-type tumour promoter, on arachidonic acid metabolism in rat peritoneal macrophages. Br. J. Pharmacol. 94, 917–923.

Robbins, M., McKinney, M., 1992. Transcriptional regulation of neuromodulin (GAP-43) in mouse neuroblastoma clone N1E-115 as evaluated by the RT/PCR method. Brain Res. Mol. Brain Res. 13, 83–92.

Satoh, K., Imaizumi, T., Yoshida, H., Takamatsu, S., 1993. Effect of 17β-estradiol on secretion of platelet-activating factor acetylhydrolase by HepG2 cells. Metabolism 42, 672–677.

Shakhov, A.N., Collart, M.A., Vassalli, P., Nedospasov, S.A., Jongeneel, C.V., 1990. Kappa B-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor alpha gene in primary macrophages. J. Exp. Med. 171, 35–47.

Shimizu, H., Mitomo, K., Watanabe, T., Okamoto, S., Yamamoto, K., 1990. Involvement of a NF-κB-like transcription factor in the activation of the inerleukin-6 gene by inflammatory lymphokines. Mol. Cell. Biol. 10, 561–568.

Stewart, A.G., Phillips, W.A., 1989. Intracellular platelet-activating factor regulates eicosanoid generation in guinea-pig resident peritoneal macrophages. Br. J. Pharmacol. 98, 141–148.

Sugiura, T., Fukuda, T., Masuzawa, Y., Waku, K., 1990. Ether lysophospholipid-induced production of platelet-activating factor in human polymorphonuclear leukocytes. Biochim. Biophys. Acta 1047, 223–232.

Tada, H., Shiho, O., Kuroshima, K., Koyama, M., Tsukamoto, K., 1986. An improved colorimetric assay for interleukin 2. J. Immunol. Methods 93, 157–165.

Tessner, T.G., Greene, D.G., Wykle, R.L., 1990. Selective deacylation of arachidonate-containing ethanolamine-linked phosphoglycerides in stimulated human neutrophils. J. Biol. Chem. 265, 21032–21038.

Thastrup, O., Foder, B., Scharff, O., 1987. The calcium mobilizing tumor promoting agent, thapsigargin elevates the platelet cytoplasmic free calcium concentration to a higher steady state level. A possible mechanism of action for the tumor promotion. Biochem. Biophys. Res. Commun. 142, 654–660.

Tsunoda, H., Sakuma, Y., Harada, K., Muramoto, K., Katayama, S., Horie, T., Shimomura, N., Clark, R., Miyazawa, S., Okano, K., 1990. Pharmacological activities of a novel thienodiazepine derivative as a platelet-activating factor antagonist. Arzneim.-Forsch. 40, 1201–1205.

Uemura, Y., Lee, T.C., Snyder, F., 1991. A coenzyme A-independent transacylase is linked to the formation of platelet-activating factor (PAF) by generating the lyso-PAF intermediate in the remodeling pathway. J. Biol. Chem. 266, 8268–8272.

Van Snick, J., 1990. Interleukin-6: an overview. Annu. Rev. Immunol. 8, 253–278.

Van Zaanen, H.C., Koopmans, R.P., Aarden, L.A., Rensink, H.J., Stouthard, J.M., Warnaar, S.O., Lokhorst, H.M., van Oers, M.H., 1996. Endogenous interleukin 6 production in multiple myeloma patients treated with chimeric monoclonal anti-IL6 antibodies indicates the existence of a positive feed-back loop. J. Clin. Invest. 98, 1441–1448.

Venable, M.E., Nieto, M.L., Schmitt, J.D., Wykle, R.L., 1991. Conversion of 1-*O*-[^{3}H]-alkyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine to lyso platelet-activating factor by the CoA-independent transacylase in membrane fractions of human neutrophils. J. Biol. Chem. 266, 18691–18698.

Watanabe, M., Watanabe, T., Hirasawa, N., Mue, S., Muramatsu, C., Matsushita, Y., Takahagi, H., Braquet, P., Broquet, C., Levine, L., Ohuchi, K., 1992. Preparation of immunoaffinity mini-columns for the analysis of platelet activating factor (PAF) in biological samples. J. Chromatogr. 597, 309–314.

Watanabe, M., Arakida, Y., Tanabe, J., Sugidachi, A., Hirasawa, N., Mue, S., Ohuchi, K., 1994. Pharmacological analysis of neutrophil chemotactic factor production by leucocytes and roles of PAF in allergic inflammation in rats. Br. J. Pharmacol. 111, 123–130.

Watanabe, M., Yamada, M., Mue, S., Ohuchi, K., 1995. Enhancement by cyclo-oxygenase inhibitors of platelet-activating factor production in thapsigargin-stimulated macrophages. Br. J. Pharmacol. 116, 2141–2147.

Winkler, J.D., Sung, C.M., Bennett, C.F., Chilton, F.H., 1991. Characterization of CoA-independent transacylase activity in U937 cells. Biochim. Biophys. Acta 1081, 339–346.

Winkler, J.D., Sung, C.M., Hubbard, W.C., Chilton, F.H., 1992. Evidence for different mechanisms involved in the formation of lyso platelet-activating factor and the calcium-dependent release of arachidonic acid from human neutrophils. Biochem. Pharmacol. 44, 2055–2066.

Winkler, J.D., Fonteh, A.N., Sung, C.M., Heravi, J.D., Nixon, A.B., Chabot-Fletcher, M., Griswold, D., Marshall, L.A., Chilton, F.H., 1995. Effects of CoA-independent transacylase inhibitors on the production of lipid inflammatory mediators. J. Pharmacol. Exp. Ther. 274, 1338–1347.

Yamada, M., Watanabe, M., Mue, S., Ohuchi, K., 1996. Platelet-activating factor production in stimulated macrophages is down-regulated by concurrently produced prostaglandin E_2. J. Pharmacol. Exp. Ther. 277, 1607–1614.

Yamada, M., Ichinowatari, G., Tanimoto, A., Yaginuma, H., Ohuchi, K., 1998. Inhibition of tumor necrosis factor-α production by SK&F 98625, a CoA-independent transacylase inhibitor, in cultured rat peritoneal macrophages. Life Sci. 62, 297–302.

Yamada, M., Tanimoto, A., Ichinowatari, G., Yaginuma, H., Ohuchi, K., 1999. Possible participation of intracellular platelet-activating factor in tumor necrosis factor-α production by rat peritoneal macrophages. Eur. J. Pharmacol. 374, 341–350.

Yoshida, S., Kato, T., Sakurada, S., Kurono, C., Yang, J.P., Matsui, N., Soji, T., Okamoto, T., 1999. Inhibition of IL-6 and IL-8 induction from cultured rheumatoid synovial fibloblasts by treatment with aurothioglucose. Int. Immunol. 11, 151–158.

You, M., Flick, L.M., Yu, D., Feng, G.S., 2001. Modulation of the nuclear factor κB pathway by Shp-2 tyrosine phosphatase in mediating the induction of interleukin (IL)-6 by IL-1 or tumor necrosis factor. J. Exp. Med. 193, 101–110.