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Study on the Action by PAF on IL-1 Modulation in Alveolar Macrophages: Involvement of Endogenous Arachidonate Metabolites and Intracellular Ca^{++} Mobilization

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Platelet-activating factor(PAF) enhanced interleukin-1(IL-1) activity by the interaction with a specific receptor in rat alveolar macrophages. In this study, we investigated the role of endogenous arachidonate metabolites and intracellular calcium mobilization in the PAF-induced IL-1 activity. Alveolar macrophages were preincubated with 5-lipoxygenase and cyclooxygenase inhibitors 30 min before the addition of PAF and lipopolysaccharide(LPS). After 24h culture, IL-1 activity was measured in the supernate of sample using the thymocyte proliferation assay. Inhibition of 5-lipoxygenase by nordihydroguaiaretic acid and AA-861 completely blocked the PAF-induced enhancement of IL-1 activity with IC_{50} of 2 μM and 5 μM, respectively. In contrast, the inhibition of cyclooxygenase pathway by indomethacin and ibuprofen resulted in the potentiation in PAF-induced IL-1 activity with maximal effect at 1 μM and 5 μM, respectively. In addition, leukotriene B_4 and prostaglandin E_2 production were observed in PAF-stimulated alveolar macrophage culture. As could be expected, 5-lipoxygenase and cyclooxygenase inhibitors abolished PAF-stimulated leukotriene B_4 and prostaglandin E_2 production, respectively. The effects of PAF on intracellular calcium mobilization in alveolar macrophages were evaluated using the calcium-sensitive dye fura-2 at the single cell level. PAF at any dose between 10^{-16} and 10^{-8} M did not increase intracellular calcium. Furthermore, there was no effective change of intracellular calcium level when PAF was added to alveolar macrophages in the presence of LPS or LPS+LTB_4, and 4, 24 and 48h after treatment of these stimulants. Together, the results indicate that IL-1 activity induced by PAF is differently regulated through subsequent induction of endogenous 5-lipoxygenase and cyclooxygenase pathways, but not dependent on calcium signalling pathway.

Key Words: PAF, IL-1, Alveolar macrophages, Lipoxygenase inhibitors, Cyclooxygenase inhibitors, Leukotriene B_4, Prostaglandin E_2, Intracellular Ca^{++} mobilization

INTRODUCTION

Platelet-activating factor (PAF) has been recently described as a potent mediator in inflammatory and immunologic reaction since it is produced by a variety of cells including neutrophils, monocytes-macrophages, basophils, endothelial cells, platelets, and eosinophils (Chung, 1992). After stimulation with various allergic and nonallergic stimulants, released PAF exhibits various and potent biological effects on its target cells or tissues. In macrophages, PAF enhances the release of arachidonic acid and the production of various eicosanoids (Chao et al, 1989) as well as pro-inflammatory cytokines such as interleukin-1 (Lee, 1997), TNF (Lee & Han, 1996) and interleukin-6 (Thivierge & Rola-Pleszcznski, 1992). As well as enhancing the production of these biological active mediators, PAF may play a role as a initiating or priming mediator among a network including these lipid and protein mediators associated with inflammation and tissue injury (Dubois et al, 1989).

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Numerous studies have indicated that PAF induces its various effects on target cells through interaction with specific receptors (Hwang et al, 1983). With the use of [^{3}H]PAF as a radioligand, high affinity binding sites on human and rabbit platelets (Valone et al, 1982; Inarrea et al, 1984), human neutrophils (O'Flaherty et al, 1986), and murine and rat macrophages (Liu et al, 1992)have been demonstrated. Upon binding to its receptor, PAF initiates the signal events, including stimulation phospholipase A_2 and phospholipase C through G-proteins (Chao et al, 1993). Therefore, the release of arachidonic acid, and production of eicosanoids and the hydrolysis of phosphatidylinositol 4,5-bisphosphate yielding two second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($InsP_3$) in response to PAF were resulted in (Hwang, 1990). DAG was found to activate protein kinase C leading to phosphorylation of various substrates whereas $InsP_3$ mobilizes intracellular calcium (Berridge et al, 1984).

In previous experiments, PAF was found to enhance IL-1 activity by rat alveolar macrophages in the presence of muramyl dipeptide (MDP), lipopolysaccharide (LPS) or silica by 2-3 fold above the value of cell cultures with stimulant alone (Lee, 1997). This enhancement of IL-1 activity by PAF was almost blocked by PAF-specific receptor antagonists such as BN 52021 and CV 3988, suggesting that the PAF action on IL-1 activity may be mediated through interaction with a specific receptor. The production of arachidonic acid metabolites and intracellular calcium mobilization induced by PAF has been considered to be associated with some biological effects of PAF (Camussi et al, 1990; Chao & Olson, 1993). Therefore, in the present study, we investigated first, the role of endogenous lipoxygenase and cyclooxygenase metabolites and second, the characterization of intracellular calcium mobilization in the PAF-stimulated IL-1 activity by alveolar macrophages.

METHODS

Chemical reagents

PAF (Sigma Co., MO, USA) was dissolved in ethanol and suspended in phosphate-buffered solution (PBS) containing 2.5% bovine serum albumin. The lipoxygenase inhibitors such as NDGA (Sigma Co., MO, USA) and AA-861 (Biomol, PA, USA), and the cyclooxygenase inhibitors such as indomethacin (Sigma Co., MD, USA) and ibuprofen (Biomol, PA, USA) were dissolved in ethanol and methanol, respectively, and further diluted with RPMI 1640. Final concentrations of ethanol and methanol in cell culture were less than 0.01%. Lipopolysaccharide from E.Coli serotype 055B5 was obtained from Sigma. Fura-2 was purchased from Molecular Probes (OR, USA).

Cell culture and supernate preparation

Alveolar macrophages were obtained from specific pathogen free male Sprague-Dawely rats (250~280 g) (Sweeney et al, 1981). Briefly, rats were anesthetized by intraperitoneal injection of secobarbital sodium (60 mg/kg body weight). The trachea was then cannulated and the lungs were lavaged 10 times with 8 ml aliquots of Ca^{++}, Mg^{++} free Hanks balanced salt solution (145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4 and 5.5 mM glucose, pH=7.4). Cells were washed with the same buffer solution and cell numbers, purity, and volume were measured using an electronic coulter counter with a cell sizing channelyzer (Coulter Electronics, Beds, England). Cells were then suspended in RPMI-1640 media with 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 units/ml nystatin, and 10% heat-inactivated fetal calf serum. Aliquots of 1 ml containing 10^6 alveolar macrophages were added to 24 well plates (Costar, MA, USA) and incubated for 2h at 37°C in a humidified atmosphere of 5% CO_2. The nonadherent cells were then removed with two 1 ml washes of the fresh RPMI media. The adherent cells were pretreated with lipoxygenase inhibitor (1~50 μM) or cyclooxygenase inhibitor (0.5~10 μM) in 1 ml of the RPMI media 30 min prior to the addition of PAF (10^{-14} M) and LPS (1.0 μg/ml). After incubating for 24h, cell cultures were centrifuged at 500 g for 15 min and the supernates frozen at −70°C until assayed.

Measurement of IL-1 activity

Alveolar macrophage supernates were assayed for their IL-1 activity by using the mouse thymocyte proliferation assay according to the method of Lackman et al (1980). Briefly, thymocytes were obtained from male ICR mice (4~8 weeks of age) and suspended in RPMI-1640 media with 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomy-

cin, 100 units/ml nystatin, 10% heat-inactivated fetal calf serum, and 2×10^{-5} M mercaptoethanol. Cells were counted using an electronic counter and adjusted to a concentration of 10×10^{6} cells/ml. An aliquot of 100 μl of the macrophage-conditioned supernates was placed in quadruplicate to 96-well microculture plates and 100 μl of thymocyte suspension was added in each well. Cultures were incubated for 48h in humidified CO_2 at 37°C, pulsed for 6h with [^{3}H] thymidine (1.0 μCi/well, activity: 2.0 Ci/mmol, Dupont NEN Products, MA, USA), and harvested onto glass fiber filters with a cell harvester (Brandle M-12, MD, USA). Total cell-associated radioactivity was measured using a Beckman liquid scintillation counter (Model 6500, France). The levels of IL-1 activity in the tested macrophage supernates were expressed as counts per minute and then calculated as a percentage of the unstimulated control sample response.

Leukotriene B4 and prostaglandin E2 assay

Alveolar macrophages (1×10^{6} cells/ml) were incubated in RPMI media in the presence or absence of PAF, lipoxygenase inhibitors, or cyclooxygenase inhibitors either alone or in combination. After 24h incubation, supernates were collected and assayed for LTB_4 or PGE_2 contents using a commercial enzyme-linked immunoassay (Amersham, UK). LTB_4 and PGE_2 values are expressed as pg/1×10^{6} cells.

Measurements of intracellular Ca^{++} mobilization

Intracellular Ca^{++} mobilization was measured using a Ca^{++} sensitive indicator dye Fura-2 (Kim & Rabin, 1994). Cells were inoculated onto coverglass (5×10^{6} cells/chamber) incubated for 2h at 37°C. The cells were washed twice with KRH (Krebs-Ringer buffer; 10 mM Hepes, 125 mM NaCl, 5 mM KCl, 1.0 mM $MgSO_4$, 10 mM sodium phosphate, 5 mM glucose, 1.3 mM $CaCl_2$, and 0.25% bovine serum albumin, pH 7.4) and incubated with 10 μM Fura-2 at 37°C for 30 min. Extracellular dye was then removed by washing the cells three times and 1 ml of KRH added. Ca^{++}-dependent fluorescence in cells was quantified on Photon technology Image (PTI, USA).

Data analysis

Data were expressed as means±standard errors of separate experiments. Statistical significance was determined using a Student t-test with significance set at $p<0.05$ or <0.01.

RESULTS

We previously found that PAF significantly enhanced IL-1 activity by rat alveolar macrophages in the presence of MDP and LPS ($p<0.01$)(Fig. 1). This effect of PAF was markedly abolished PAF-specific receptor antagonists such as BN 52021 and CV 3988, suggesting that the PAF action on IL-1 activity may be mediated through interaction with its specific receptor (Fig. 2). In the present study, first, the role of endogenous lipoxygenase and cyclooxygenase metabolites in IL-1 activity by PAF-stimulated alveolar macrophges was investigated. Lipoxygenase inhibitors such as NDGA and AA861, and cyclooxygenase inhibitors such as indomethacin and ibuprofen were treated for 30 min in alveolar macrophages before the addition of PAF with LPS. Fig. 3 shows that NDGA (5 μM), a nonspecific lipoxygenase inhibitor and AA-861 (5 μM), specific 5-lipoxygenase inhibitor almost blocked the enhancement of IL-1 activity induced by PAF. The basal stimulation with LPS alone also inhibited by NDGA and AA-861. In contrast, indomethacin and ibuprofen potentiated IL-1

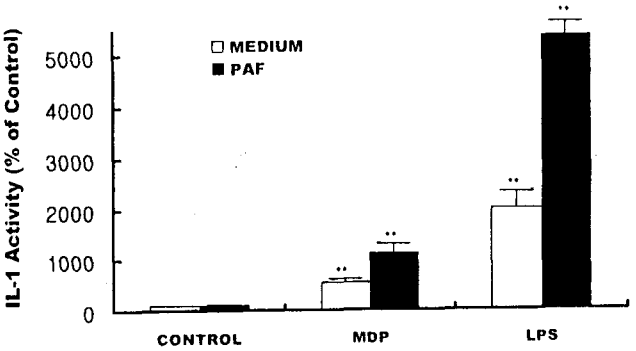

Fig. 1. Effect of PAF on MDP- and LPS-induced IL-1 activity by rat alveolar macrophages. Alveolar macrophages (1×10^{6}) were stimulated with PAF (10^{-14} M) in the presence of MDP (10 μg/ml) or LPS (1.0 μg/ml). Cell-free supernates were collected after 24h, and IL-1 activity was measured in the thymidine incorporation assay using mouse thymocytes. Data are expressed as percentage over control levels obtained from nonstimulated cells, and represent means±SEM of four experiments. **$p<0.01$ for stimulant- or stimulant+PAF-stimulated cells compared with unstimulated cells.

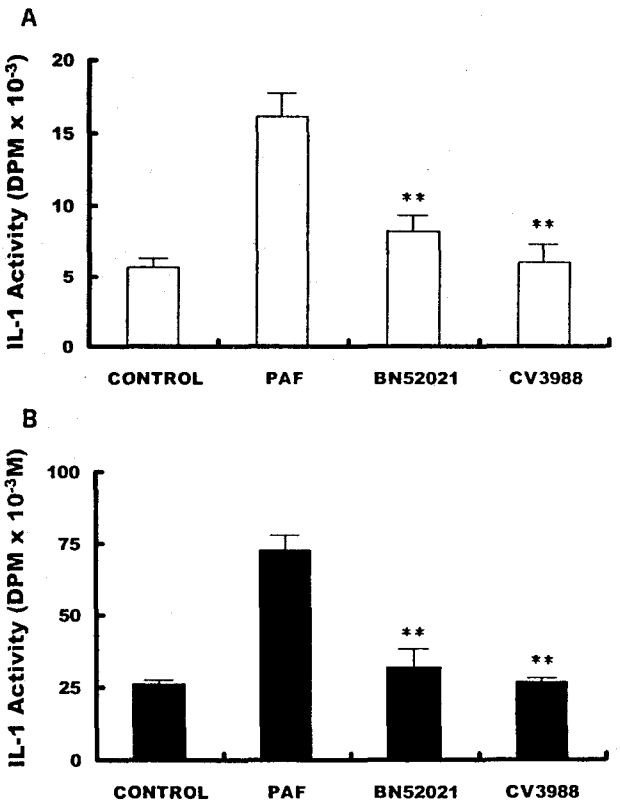

Fig. 2. Effect of specific PAF-receptor antagonist on IL-1 activity by rat alveolar macrophages. Alveolar macrophages (1×10^6) were preincubated with BN 52021 (10^{-5} M) or CV 3988 (10^{-5} M) for 15 min at 37°C and then stimulated with PAF in the presence of MDP (Fig. 2A) or LPS (Fig. 2B). After 24h incubation, IL-1 activity in the supernates was measured using thymocyte proliferation assay. Data present means±SEM of four experiments. *$p<0.01$ for PAF antagonist-pretreated cells in the presence of MDP+ or LPS+ PAF compared with MDP+ or LPS+PAF stimulated cells.

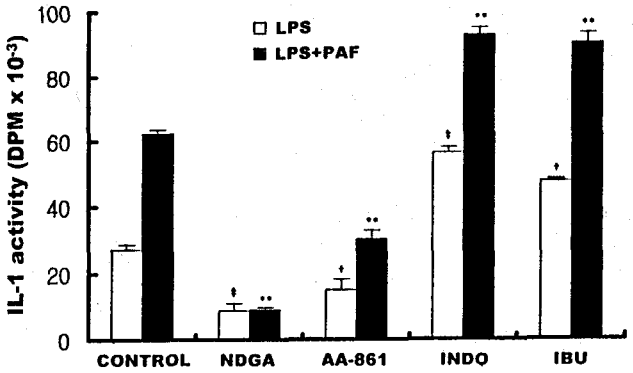

Fig. 3. Effect of lipoxygenase and cyclooxygenase inhibitors on PAF-enhanced IL-1 activity. Alveolar macrophages (1×10^6) were preincubated 30 min with AA 861, NDGA (5 μM), indomethacin, or ibuprofen(1 μM) before addition of LPS (1.0 μg/ml) and PAF (10^{-14} M). After 24h incubation, IL-1 activity in the supernates was measured using thymocytes proliferation assay. Data are expressed as percentage over the values obtained from nonstimulated cells, and represent means±SEM of four experiments. **$p<0.01$ for lipoxygenase or cyclooxygenase inhibitor- pretreated cells in the presence of LPS+PAF compared with control levels obtained from LPS+PAF stimulated cells while differences between lipoxygenase or cyclooxygenase inhibitor-pretreated cells in the presence of LPS and LPS stimulated cells are indicated by † $p<0.05$ and ‡ $p<0.01$.

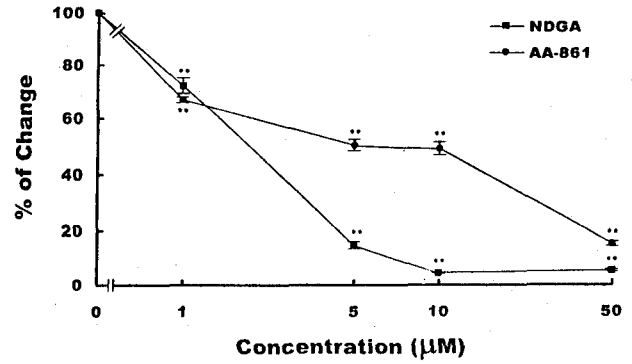

Fig. 4. Inhibition of PAF-induced IL-1 activity by graded concentrations of lipoxygenase inhibitors. Alveolar macrophages (1×10^6) were preincubated 30 min with AA 861 or NDGA (1~50 μM) and further incubated for 24h in the presence of LPS (1.0 μg/ml) and PAF (10^{-14} M). IL-1 activity in the supernates was measured using thymocytes proliferation assay. Data are expressed as percentage over the values obtained from nonstimulated cells, and represent means±SEM of four experiments. **$p<0.01$ for lipoxygenase inhibitor-pretreated cells in the presence of LPS+PAF compared with control levels obtained from LPS+PAF stimulated cells.

activity by PAF-stimulated cells in the presence of LPS as well as by AM with LPS alone. Both NDGA and AA-861, as shown in Fig. 4, exhibited dose-dependent inhibitory effects on PAF-stimulated IL-1 activity. Maximal inhibition was approximately 95% at 50 μM NDGA and 85% at 50 μM AA-861. The ID_{50} values for NDGA and AA-861 were 2 μM and 5 μM, respectively. Dose response of PAF-stimulated IL-1 activity was also examined with respect to cyclooxygenase inhibitors. Both cyclooxygenase inhibitors, indomethacin and ibuprofen resulted in increase in PAF-stimulated IL-1 activity with dose-dependent patterns (Fig. 5). The maximal increase of 195% was shown at 1 μM of indomethacin and 5 μM

of ibuprofen.

Since leukotriene B_4, a major lipoxygenase metabolite has been shown to enhance IL-1 production by human monocytes (Rola-Pleszczynski & Lemaire, 1985), but prostaglandin E_2, a major cyclooxygenase metabolite, to decrease it by peritoneal macrophages (Kunkel et al, 1986), the effects of PAF on LTB_4 and PGE_2 production by alveolar macrophages in the presence of LPS were investigated. PAF stimulated both LTB_4 and PGE_2 production with 43% and 49% above the level of basal LPS stimulation (Table 1 and 2). As could be expected, both lipoxygenase inhibitors and cyclooxygenase inhibitors effectively abolished the PAF-stimulated LTB_4 and PGE_2 production by alveolar macrophages, respectively.

To study characterization of Ca^{++} mobilization in

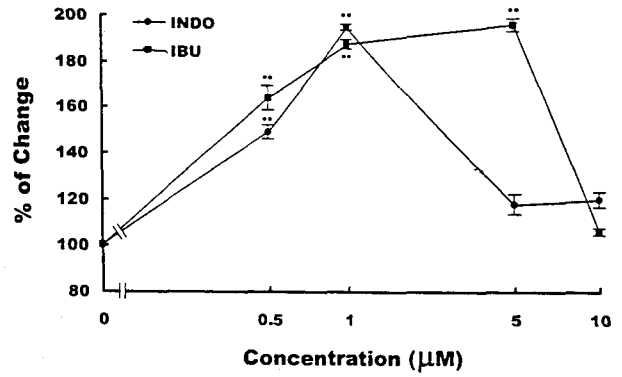

Fig. 5. Potentiation of PAF-induced IL-1 activity by graded concentrations of cyclooxygenase inhibitors. Alveolar macrophages (1×10^6) were preincubated 30 min with indomethacin or ibuprofen (0.5~10 μM) and further incubated for 24h in the presence of LPS (1.0 μg/ml) and PAF (10^{-14} M). IL-1 activity in the supernates was measured using thymocytes proliferation assay. Data are expressed as percentage over the values obtained from nonstimulated cells, and represent means±SEM of four experiments. **$p<0.01$ for cyclooxygenase inhibitor-pretreated cells in the presence of LPS+PAF compared with control levels obtained from LPS+PAF stimulated cells.

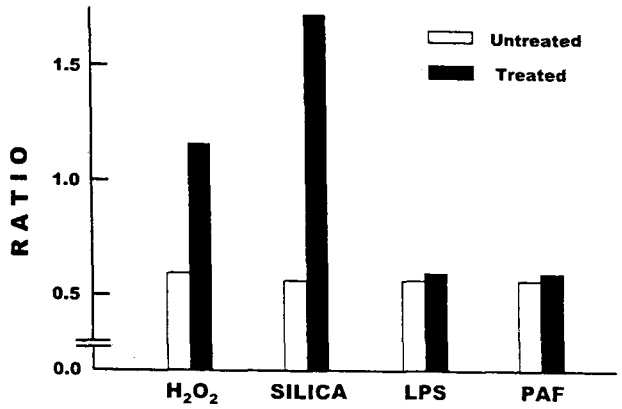

Fig. 6. Effect of PAF on calcium mobilization in alveolar macrophages. Intracellular calcium level was determined using calcium sensitive dye fura-2. After establishing a stable base line, H_2O_2 (200 μM), silica (1 mg/ml), LPS (1.0 μg/ml), or PAF (10^{-14} M) was added to the cells. Calcium mobilization is indicated by an increase in the fura-2 fluorescence ratio (F340/F380). Data represents the average change in ratio in a field containing 10~20 cells.

Table 1. Effect of lipoxygenase inhibitors on PAF-induced LTB_4 production by rat alveolar macrophages

Culture conditions	LTB_4 (pg/10^6 cells)
CONTROL	101.3
PAF	144.2
NDGA	82.0
NDGA+PAF	79.8
AA-861	87.8
AA-861+PAF	100.1

Data are given from an experiment representative of two experiments, in which alveolar macrophages(1×10^6) were preincubated or not with NDGA(5 μM) or AA-861(5 μM) then cultured for 24h with PAF(10^{-14} M) and LPS(1.0 μg/ml). LTB_4 was detected by enzyme-linked immunoassay.

Table 2. Effect of cyclooxygenase inhibitors on PAF- induced PGE_2 production by rat alveolar macrophages

Culture conditions	PGE_2 (pg/10^6 cells)
CONTROL	104.1
PAF	155.6
INDO	96.6
INDO+PAF	79.6
IBU	99.9
IBU+PAF	77.8

Data are given from an experiment representative of two experiments, in which alveolar macrophages(1×10^6) were preincubated or not with indomethacin(1 μM) or ibuprofen(1 μM) then cultured for 24h with PAF(10^{-14} M) and LPS(1.0 μg/ml). LTB_4 was detected by enzyme-linked immunoassay.

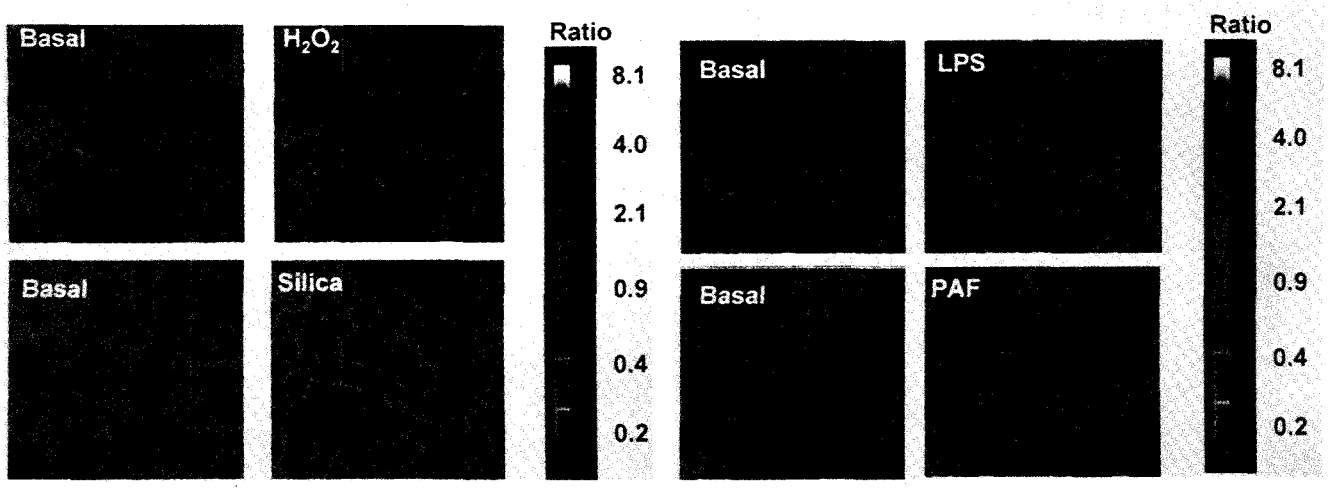

Fig. 7. Fluorescence image analysis of calcium mobilization in alveolar macrophages. Intracellular calcium level was determined using calcium sensitive dye fura-2. After establishing a stable base line, H_2O_2 (200 μM), silica (1 mg/ml), LPS (1.0 μg/ml), or PAF (10^{-14} M) was added to the cells. Fluorescent figures show maximal calcium levels in the macrophages. The color bar represents the fluorescence ratio (F340/F380).

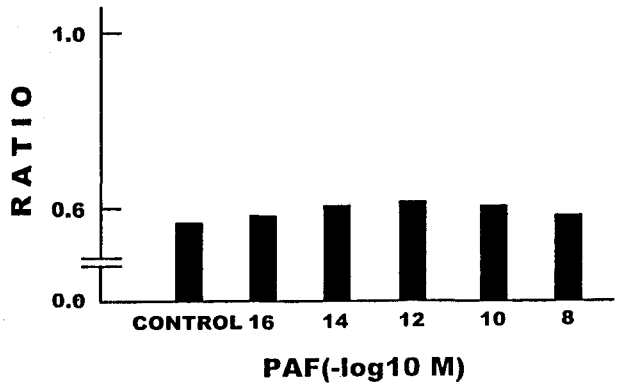

Fig. 8. Effect of various concentrations of PAF on calcium mobilization in alveolar macrophages. Intracellular calcium level was determined using calcium sensitive dye fura-2. After establishing a stable base line, graded concentration of PAF from 10^{-16} M to 10^{-8} M was added to the cells. Calcium mobilization is indicated by an increase in the fura-2 fluorescence ratio (F340/F380). Data represents the average change in ratio in a field containing 10~20 cells and an experiment representative of three experiments.

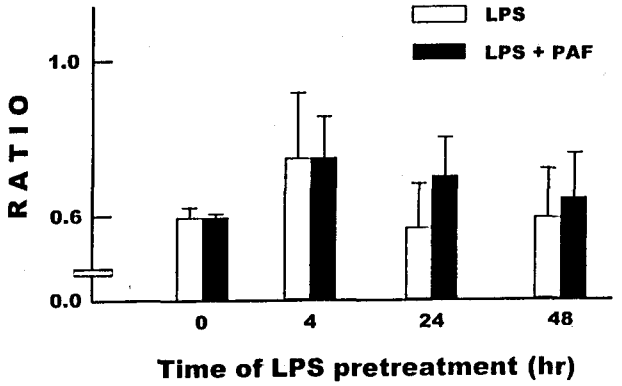

Fig. 9. Effect of various concentrations of PAF on calcium mobilization in LPS-primed alveolar macrophages. Cells were pretreated with LPS for indicated time periods and intracellular calcium levels analysed using calcium sensitive dye fura-2. After establishing a stable base line, PAF (10^{-14} M) was added to the cells. Calcium mobilization is indicated by an increase in the fura-2 fluorescence ratio (F340/F380). Data represents the average change in ratio in a field containing 10~20 cells and an experiment representative of three experiments.

PAF-stimulated alveolar macrophages, alveolar macrophages were loaded with fura-2 and the fluorescence was subsequently monitored over 10 min. Basal level of intracellular Ca^{++} of alveolar macrophages ranged from 30 to 40 nM. H_2O_2 and silica were used as positive controls since these have been known to increase Ca^{++} mobilization from the intracellular store and Ca^{++} influx through plasma membrane, respectively. As expected, the intracellular Ca^{++} level in H_2O_2- or silica-treated cells was increased above the basal level from nontreated cells as shown by the increase of the ratio between two excitation wavelengths, 340 and 380 nm (Fig. 6, 7). However, 1.0 μg/ml LPS as well as 10^{-14} M PAF at which doses the optimal increase of IL-1 activity by alveolar macrophages has been shown in our pre-

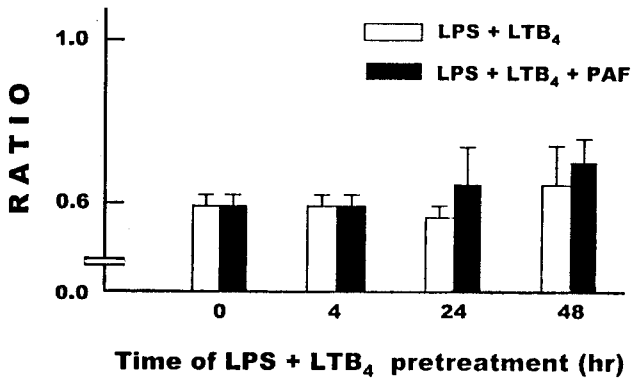

Fig. 10. Effect of various concentrations of PAF on calcium mobilization in LPS+LTB_4 primed alveolar macrophages. Cells were treated with LPS (1.0 μg/ml)+ LTB_4 (10^{-8} M) for indicated time periods and analyzed intracellular calcium level using calcium sensitive dye fura-2. After establishing a stable base line, PAF (10^{-14} M) was added to the cells. Calcium mobilization is indicated by an increase in the fura-2 fluorescence ratio (F340/380). Data represents the average change in ratio in a field containing 10~20 cells and an experiment representative of two or three experiments.

vious experiments (Lee, 1997), had no effect on Ca^{++} mobilization (Fig. 6, 7). Unresponse of alveolar macrophages to LPS or PAF has been exhibited over 30 min after treatment of LPS or PAF (data not shown). Even higher concentrations of PAF also did not change the intracellular Ca^{++} level (Fig. 8). To investigate the effect of PAF on intracellular Ca^{++} mobilization by LPS-primed alveolar macrophages, cells were incubated with LPS for 0, 4, 24 and 48h and intracellular Ca^{++} levels monitored subsequently after addition of PAF (10^{-14} M). PAF failed to substantially increase in intracellular Ca^{++} levels from the primed cells with LPS (Fig. 9). As shown in the primed-cells with LPS, there were no effective changes of intracellular Ca^{++} levels in PAF-stimulated alveolar macrophages primed with LPS and LTB_4 together for 4~48h (Fig. 10).

DISCUSSION

Interleukin-1 is a major cytokine released by macrophages/monocytes, which has potent amplifying effects on the inflammatory response. In addition, IL-1 plays important role in the initiation of an immune response through its direct activation of lymphocytes and stimulation of the production of other cytokines (Dinarello, 1989). The regulation of IL-1 production, therefore, may be essential to the ultimate modulation of inflammatory and immunologic responses.

In the previous study, PAF has been shown to enhance IL-1 activity by rat alveolar macrophages and its specific antagonists blocked this effect (Lee, 1997). Endogenous arachidonic acid metabolites as well as intracellular calcium have been suggested secondary messengers in the underlying mechanism of PAF action (Thivierge & Rola-Pleszczynski, 1989; Dubois et al, 1989).

The present study indicates that PAF induces both up- and down-regulation through 5-lipoxygenase pathway and cyclooxygenase pathway, respectively, in its interaction with alveolar macrophages and IL-1 production. This evidence for the interelationship between the biological effect of PAF and the endogenous 5-lipoxygenase products has been also presented in TNF production by PAF-stimulated rat alveolar macrophages (Thivierge & Rola-Pleszczynski, 1993). LTB_4, a major 5-lipoxygenase product of arachidonic acid has been well documented to be a powerful activator in phagocyte functions and a modulator in immunoregulatory process (Rola-Pleszczynski & Stankova, 1992). In addition, exogenous and endogenous LTB_4 have been shown to enhance various cytokine production such as IL-1 (Rola-Pleszczynski & Lemaire, 1985) and TNF_α (Thivierge & Rola-Pleszczynski, 1989) in rat alveolar macrophges. In the present study, we also observed LTB_4 production by PAF-stimulated alveolar macrophages in the presence of LPS and confirmed suppression of its production by lipoxygenase inhibitors.

On the other hand, while the inhibition of cyclooxygenase pathway further potentiated PAF-induced IL-1 activity, it suppressed the production of PGE_2, a major cytokine of cyclooxygenase metabolite in PAF-stimulated alveolar macrophages. This inhibitory involvement of the cyclooxygenase pathway in the PAF-stimulated cells was consistently shown in another cytokine production such as TNF_α (Thiverge & Rola-Pleszczynski, 1993). Furthermore, the inhibitory effect of endogenous PGE_2 on IL-1 activity has been previously reported in the LPS-induced IL-1 production from mouse peritoneal macrophages (Kunkel et al, 1986). Our results of PAF-induced PGE_2 production in rat alveolar macrophages in the presence of LPS are supported by the studies of Beusenberg et al (1994) and Thiverge & Rola- Pleszczynski (1993), who reported that PAF enhanced the

PGE_2 production in antigen-stimulated guinea pigs alveolar macrophages and MDP-stimulated rat alveolar macrophages, respectively. As like endogenous PGE_2, the suppressive effect of exogenous PGE_2 on LPS-induced IL-1 production has been illuminated (Kunkel et al, 1986). These findings, all together, are consistent with the possibility that the action of PAF on alveolar macrophages can be modulated by the production of both endogenous 5-lipoxygenase metabolites including LTB_4 and cyclooxygenase metabolites including PGE_2, which will further act as a secondary messenger to regulate IL-1 production.

PAF has been shown to directly increase in intracellular calcium in monocytes/macrophages including human mononuclear cells (Katnik & Nelson 1993), human alveolar macrophages (Schberg et al, 1991), mouse peritoneal macrophages (Conad & Rink, 1986) as well as monocyte-macrophages like cells (Weber et al, 1991, Asmis et al, 1994; Aepfelbacher et al, 1992). In contrast, we found that PAF at various concentrations from 10^{-16} to 10^{-8} M had no effect on calcium mobilization in rat alveolar macrophages. Our findings are in accordance with results from Pendino et al (1993), who presented that 10^{-12} M PAF failed to induce intracellular calcium mobilization in the rat alveolar macrophages, suggesting PAF action on intracellular calcium mobilization to be species-specific and providing correlation with our previous findings of unresponse of rat alveolar macrophages to PAF alone without stimulant in associated with the release of reactive oxygen species (Lee, 1995), production of IL-1 (Lee, 1997) and TNF_α (Lee & Hah, 1996). Furthermore, in our experiments, PAF could not substantially increase intracellular calcium level in alveolar macrophages in the presence of LPS or LPS+LTB_4 as well as primed cells with these stimulants for 4~48h whereas in the study of Pendino et al (1993), PAF caused a rapid and transient rise in intracellular calcium in alveolar macrophages obtained from rats exposed to ozone. This discrepancy may be described by different experimental mode between in vitro and in vivo exposure, supporting the concept that macrophages develop specialized functions in response to their microenvironment.

In conclusion, the present results indicate that IL-1 activity induced by PAF-stimulated alveolar macrophages is differently regulated by subsequent induction of the endogenous 5-lipoxygenase and cyclooxygenase pathway, but not dependent on the calcium signalling pathway.

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