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Platelet-Activating Factor Enhances Interleukin-1 Activity by Alveolar Macrophages : Inhibition by PAF Specific Receptor Antagonists

Jihee Lee

Department of Physiology, College of Medicine, Ewha Womans University and Division of Cell Biology, Ewha Medical Research Center 911-1 Mok-6-dong, Yangcheonku, Seoul 158–056, Korea

It is becoming increasingly clear that the inflammatory reaction can be ascribed to a complex array of mediators generated and released from activated phagocytes. In this study, the effect of PAF on interleukin-1(IL-1) activity by rat alveolar macrophages(AM) was examined using thymocyte proliferation assay in the supernate of sample obtained after 24 hr culture. When AM were cultured with PAF alone, no change in IL-1 activity was observed. However, the combined addition of PAF and muramyl dipeptide(MDP) or lipopolysaccharide(LPS) to AM cultures markedly enhanced IL-1 activity by 2-3 fold compared with AM cultures with the stimulant alone in a concentration dependent fashion. The peack effect was found at 10^{-8} M PAF with MDP and 10^{-14} M PAF with LPS. the effect of PAF was also tested in silica, toxic respirable dust, -added AM cultures as well as in the cultures containing bacterial compounds. Although silica did not stimulate the IL-1 activity, PAF could enhance IL-1 activity by 2 fold above the value of the silica-treated AM cultures with the peak response at 10^{-12} M PAF. Optimal enhancement of IL-1 activity occured when MDP and PAF were present together at the initiation of the 24 hr AM cultures. Additionaly, the biologically inactive precursor/metabolite of PAF, lyso-PAF failed to induce enhancement of IL-1 activity. When the specific, but structurally different PAF receptor antagonists, BN 52021(10^{-5} M) and CV 3988(10^{-5} M) was treated 15 min before addition of PAF(10^{-8} M) and MDP(10 μg/ml) to the AM cultures, it markedly inhibited the enhancement of IL-1 activity induced by PAF. The effects of these PAF antagonists were also observed in LPS(1.0 μg/ml)-stimulated cells. Collectively, these data suggest that PAF enhances IL-1 activity by interaction with a specific receptor.

Key Words: PAF, IL-1, Alveolar macrophages, PAF antagonists, Lyso-PAF

INTRODUCTION

It is becoming clear that injury to cells or tissues related to the inflammatory reaction can be ascribed to a complex array of mediators produced from activated phagocytes. Alveolar macrophages(AM) play a important role in the first defenses of lungs and brunchus against respirable dust and microbes and in the onset and the development of both inflammatory and immune reaction by their ability to synthesize and release inflammatory mediators such as platelet-activating factor(Rylander & Beijer, 1987), arachidonate metabolites such as leukotriene B_4(Martin et al, 1984), prostaglandin E_2(Hsueh et al, 1980), and various kinds of cytokines(King et al, 1989)

Interleukin-1(IL-1) is a major cytokine released by macrophages/monocytes, which has multiple biologic effects. It induces leukocytes(Sauder et al, 1984), endothelial cell activation(Movat et al, 1987), and promotes fibroblast proliferation(Schmidit et al, 1984), synthesis collagens(Goldring et al, 1986) and glucosaminoglycans(Bronson et al 1987), and prostaglandin E_2 production(Dayer et al, 1979). 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, such as IL-2, interferons and hemopoietic

Corresponding to: Jihee Lee, Department of Physiology, College of Medicine, Ewha Womans University and Division of Cell Biology, Ewha Medical Research Center 911-1 Mok-6-dong, Yangcheonku, Seoul 158-056, Korea

colony-stimulating factors, needed for the expression of an immune response(Dinarello, 1989). Bacterial compounds such as lipopolysaccharide(LPS) and muramyl dipeptide(MDP), phorbol myristate acetate (PMA), immune complexes, and respirable toxic dusts such as silica and asbestos are known to activate the production of IL-1 by monocytes and macrophages(Oppenheim et al, 1986). The regulation of IL-1 production may be essential to the ultimate modulation of inflammatory and immune responses. Platelet-activating factor(PAF) has been recently described as a potent mediator in the pathogenesis of lung inflammation, injury, fibrosis and immunologic reaction since it is produced by a variety of cells involved in inflammatory and immune reactions including neutrophils, monocytes-macrophages, basophils, endothelial cells, platelets, and eosinophils (Chung, 1992). After stimulation with various allergic and non-allergic stimulants, PAF exhibits wide and potent biological effects in the lungs. PAF induces isometric contraction of human bronchial smooth muscle in vitro (Johnson et al, 1990) and in vivo(Cuss et al, 1986) and an immediate increase in microvascular leakage throughout the respiratory tract in the guinea-pig indicating increase in pulmonary vascular permeability (Northover & Northover, 1993). PAF stimulates the secretion of mucus from explants of trachea of variety of species including human(Lundgren et al, 1990) and increases bronchial responsiveness to methacholine in normal subjects(Cuss et al, 1986). PAF causes transient neutrophil and eosinophil accumulation of the airways, and activates chemiluminesence in these phagocytes. In macrophages, PAF augments stimulants-induced respiratory burst, chemotaxis, and oncogene expression(Ho et al, 1987; Huang et al, 1988). Recently, PAF is considered as a mediator acting among a network of mediators involved in inflammation and tissue injury since interactions of PAF with a range of cytokines and with arachidonic acid products are illustrated(Dubois et al, 1989).

Accumulating evidence indicates that PAF induces its various effects on target cells through interaction with specific receptors(Hwang 1988). With the use of [^{3}H]PAF as a radioligand, high affinity binding sites on human and rabbits platelets(Valone et al, 1982; Inarrea et al, 1984) and human neutrophils(O'Flaherty et al, 1986) have been demonstrated. Recently, the cloning and expression of a complementary DNA for a PAF receptor from guinea-pig lung found(Honda et al, 1991).

In this study, it was investigated to determine first, whether PAF regulates IL-1 activity by rat alveolar macrophages and, second, to determine whether the action of PAF is blocked by PAF specific receptor antagonists.

METHODS

Chemical reagents

PAF and lyso-PAF (Sigma Co., MD, USA) were dissolved in ethanol and suspended in phosphate-buffered solution(PBS) containing 2.5% bovine serum albumin. The PAF-specific receptor antagonist, BN 52021(Sigma Co., MD, USA) was first dissolved in NaOH 0.5N (100 μl) following HCl 0.1N(100 μl) and diluted in PBS. CV 3988(Biomol, CA, USA) was dissolved at 50°C in PBS and cooled to room temperature before use.

AM culture and supernate preparation

Alveolar macrophages were obtained from 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 with 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 2 hr 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 further incubated in 1 ml of the RPMI media containing PAF ($10^{-16}-10^{-8}$ M) in the presence or absence MDP(10 μg/ml), LPS(1.0 μg/ml), silica(50 μg/ml), the PAF antagonists, either

alone or in combination. After incubating for 24 hr, cell culture were centrifuged at 500 g for 15 min and the supernates frozen at −70°C until they are assayed.

Measurement of IL-1 activity

Rat 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 streptomycin, 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 66 hr in humidified CO_2 at 37°C, pulsed with [^{3}H] thymidine (1.0 μCi/well, activity:2.0 Ci/mmol, Dupont NEN Products, Boston, MA), and harvested at 72 hr 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.

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

Effects of PAF on IL-1 activity by rat alveolar macrophages

When rat alveolar macrophages(AM) were initially incubated for 24hr in the presence of PAF alone, IL-1 activity was not increased at any dose of PAF from 10^{-16} to 10^{-8} M PAF(Fig. 1). However, the combined addition of PAF($10^{-16}-10^{-8}$ M) and bacterial compounds such as muramyl dipeptide(MDP) and lipopolysaccharide(LPS) to AM cultures significantly enhanced IL-1 activity by 2-3 fold compared with AM cultures with MDP or LPS alone($p<0.01$)(Fig. 1 and 2). The peak response was found at 10^{-8} M PAF with MDP and 10^{-14} M PAF with LPS. The effect of PAF was also tested in toxic respirable dust, silica-added AM cultures. Although silica did not stimulate the IL-1 activity, PAF could significantly enhance IL-1 activity by 2 fold above the value of the silica-treated AM cultures at 10^{-12} M concentration(Fig. 3).

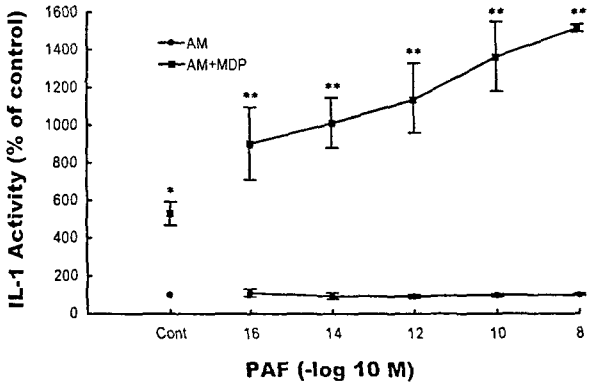

Fig. 1. Effect of PAF on MDP-induced IL-1 activity by rat alveolar macrophages.
AM(1×10^{-6} M) were stimulated with graded concentration of PAF(10^{-16}-10^{-8} M) in the presence or absence of MDP(10 μg/ml). Cell-free supernates were collected after 24 hr, and IL-1 activity was measured in the thymidine incorporation assay using mouse thymocytes. Data are expressed as percentage over control levels obtained from nonstimulated cells, and represent means±SEM of four experiments. $^{*}p<0.05$, $^{**}p<0.01$ for MDP- or MDP-+ PAF- stimulated cells compared with unstimulated cells.

Kinetics of PAF-enhanced IL-1 activity

In order to determine whether the action of PAF on IL-1 activity involves in early or late stage of IL-1 production, AM were incubated with MDP and PAF together at the beginning of the culture or cultured with MDP alone for 4 hr before addition of PAF. Optimal enhancement of IL-1 activity occured when MDP and PAF were simultaneously added(Table 1). When PAF was added 4 hr after MDP, observed at

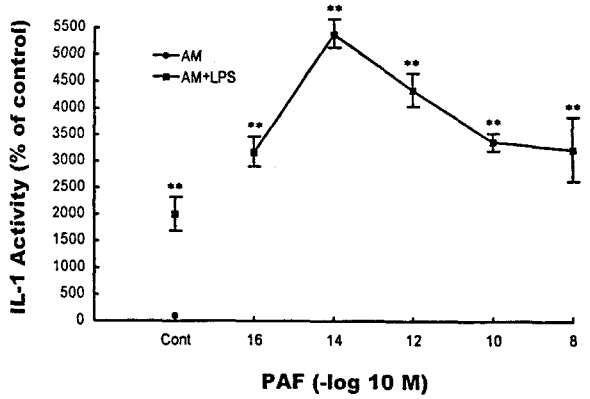

Fig. 2. Effect of PAF on LPS-induced IL-1 activity in rat alveolar macrophages.
AM(1×10^{-6} M) were stimulated with graded concentration of PAF(10^{-16}-10^{-8} M) in the presence LPS(1.0 μg/ml). Cell-free supernates were collected after 24 hr, and IL-1 activity was measured in the thymidine incorporation 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 LPS- or LPS-+PAF- stimulated cells compared with unstimulated cells.

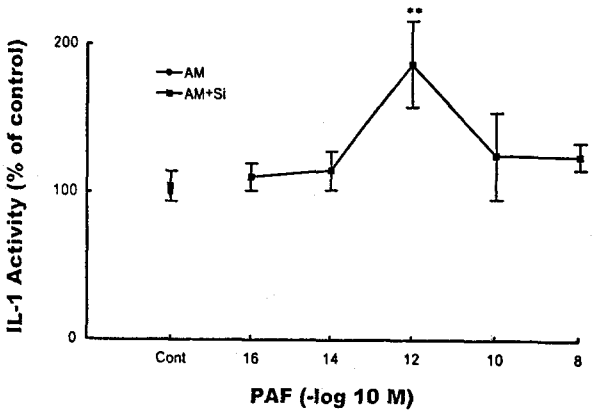

Fig. 3. Effect of PAF on silica-induced IL-1 activity by rat alveolar macrophages.
AM(1×10^{-6} M) were stimulated with graded concentration of PAF(10^{-16}-10^{-8} M) in the presence or absence of silica(50 μg/mℓ). Cell-free supernates were collected after 24 hr, and IL-1 activity measured in the thymidine 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 silica-+PAF- stimulated cells compared with unstimulated cells.

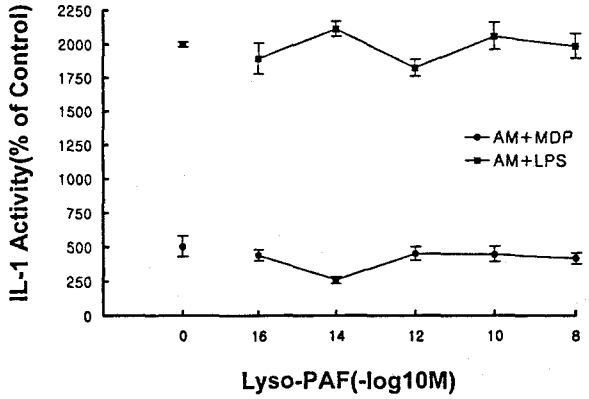

Fig. 4. Effect of lyso-PAF on IL-1 activity by rat alveolar macrophages, measured in supernates of 24 hr cultures stimulated with either MDP(10 μg/ml) or LPS(1.0 μg/ml). Data are expressed as percentage over control levels obtained from nonstimulated cells, and represent means ± SEM of four experiments.

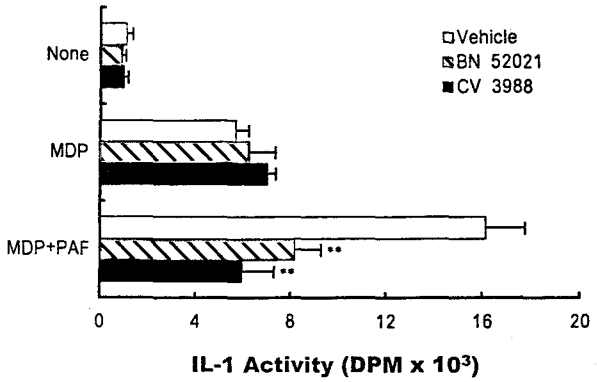

Fig. 5. Effect of specific PAF-receptor antagonist on IL-1 activity in rat alveolar macrophages. AM (1×10^{-6} M) were preincubated with BN 52021(10^{-5} M) or CV 3988 (10^{-5} M) for 15 min at 37°C and then stimulated with PAF(10^{-8} M) in the presence of MDP(10 μg/ml). After 24 hr incubation, IL-1 activity in the supernates was measured using thymocyte proliferation assay. Data represent means ± SEM of four experiments. $^{**}p<0.01$ for PAF antagonist-treated cells in the presence of MDP+ PAF compared with MDP+PAF stimulated cells.

the range of $10^{-12}-10^{-8}$ M PAF, suggesting that PAF main action occured at an early stage of IL-1 production.

Effect of lyso-PAF on IL-1 activity

Compared with the active form of PAF, the bio-

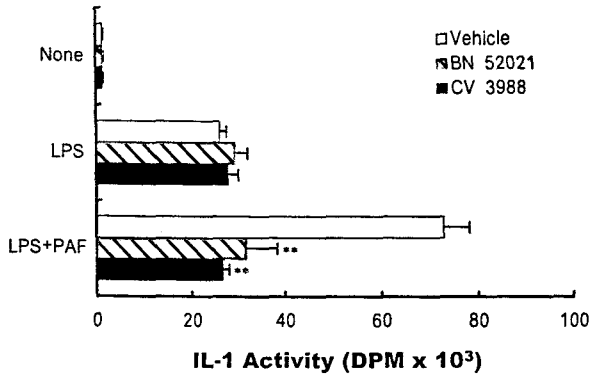

Fig. 6. Effect of specific PAF-receptor antagonist on IL-1 activity in rat alveolar macrophages. AM (1×10^{-6} M) were preincubated with BN 52021(10^{-5} M) or CV 3988 (10^{-5} M) for 15 min at 37°C and then stimulated with PAF(10^{-14} M) in the presence of LPS(1.0 μg/ml). After 24 hr incubation, IL-1 activity in the supernates was measured using thymocyte proliferation assay. Data represent means ± SEM of four experiments. $^{**}p<0.01$ for PAF antagonist-treated cells in the presence of LPS+PAF compared with LPS+PAF stimulated cells.

logically inactive PAF precursor and metabolite lyso-PAF in the range of $10^{-16}-10^{-8}$ M failed to induce significant enhancement of IL-1 activity by AM cultured with MDP or LPS(Fig. 4).

Blocking of PAF-enhanced IL-1 activity by PAF antagonists

Specific PAF-receptor antagonists were used to determine whether the effect of PAF on IL-1 activity by AM could be mediated through the specific PAF receptor. AM were preincubated for 15 min at 37°C with a PAF antagonist and then treated with MDP alone or in combination with PAF. BN 52021, a terpene derived from the Ginkgo leaf and CV 3988, a synthetic antagonist structurally analogue to PAF caused minor change in IL-1 activity in AM with or without MDP(Fig. 5). In contrast, PAF-induced enhancement of IL-1 activity was markedly blocked by BN-2021 and CV 2988. Not only in MDP-stimulated AM, effects of these specific antagonists were observed but also in LPS-stimulated cells(Fig. 6).

DISCUSSION

The present study indicates that platelet-activating

Table 1. Kinetics of Enhanced IL-1 Activity by PAF-Stimulated AM

Incubation Conditions		IL-1 Activity
0 to 4h	4-24h	(DPM ± SEM)
medium	medium	1069 ± 183
MDP	MDP	5676 ± 610
MDP	MDP(PAF 10^{-12}M)	5646 ± 622
MDP	MDP(PAF 10^{-10}M)	6535 ± 762
MDP	MDP(PAF 10^{-8}M)	9078 ± 255
MDP(PAF 10^{-12}M)	MDP	12111 ± 1926
MDP(PAF 10^{-10}M)	MDP	14502 ± 1981
MDP(PAF 10^{-8}M)	MDP	16144 ± 1604

AM(1×10^{-6} M) were incubated with MDP(10 μg/ml), and PAF(10^{-8} M) was added concomitantly or after a 4 hr delay. Cell-free supernates were collected after 24 hr, and IL-1 activity measured in the thymidineincorporation assay using mouse thymocytes. Data represent means ± SEM of four experiments.

factor(PAF) alone can not stimulate IL-1 activity by rat alveolar macrophages. However, IL-1 activity is significantly enhanced by PAF in a concentration dependent fashion compared with AM cultures with the stimulant such as MDP and LPS alone. PAF can enhances IL-1 activity in silica, toxic respirable dust, -added as well as bacterial compounds-treated AM. Up to date, this potentiative effect of PAF on rat AM stimulation has been previously reported by our lab. Lee et al(1995) showed PAF dose potentiate zymosan-stimulated activation of chemiluminescence and superoxide release. It has also recently reported that potentiative action of PAF on other cytokine production in culture of macrophage- monocyte. Pignol et al(1990) reported enhanced production of IL-1 and IL-2 in spleen cells from rats chronically infuged with PAF. Similar responses in IL-6 and TNF were obtained in rat AM in the range of $10^{-10}-10^{-8}$ M PAF with peak effect at 10^{-10} M (Thiverge & Rola-Pleszczynski, 1992). This ability of PAf to induce the potentiation of various oxidants and cytokines from macrophages-monocytes may be one of the several possible mechanisms of microbes-, immune complex-

and toxic particle-induced inflammatory and immune reaction. Not only does PAF stimulate synthesis of cytokines, but IL-1 and TNF can also induce synthesis of PAF in several cell types, including endothelial cells, neutrophils and macrophages(Valone & Epstein, 1988), suggesting the existence of a potentially powerful amplification for the contribution of the inflammatory reaction.

It must be described that not only released IL-1 activity but also intracellular IL-1 pool were measured in this study, and the similar potentiative effect of PAF was observed(the data not shown). The potentiative effect of PAF on IL-1 activity was greater when both PAF and MDP were simultaneously added together at the beginning of culture than when PAF was added 4 hr after MDP, suggesting that PAF action occurs in the early process including membrane activation, IL-1 gene expression, and new mRNA synthesis. These results on the kinetics of PAF-induced IL-1 activity are related to findings from other investigators which reported TNF production by rat alveolar macrophages(Dubois et al, 1989).

In the present study, only a very low concentration ($10^{-16} - 10^{-8}$ M) of PAF was necessary to obtain the potentiative effect of PAF on IL-1 activity, and this effect could not be resulted by PAF preccursor/metabolite lyso-PAF at the range of concentraions where PAF activity were found, suggesting a specefic effect of PAF on IL-1 activity, probably mediated by a purative receptor. It was further supported by the examination of bloking the PAF action by specific PAF antagonists. Two structually distinct PAF antagonists BN 52021, and CV 3988 resulted in inhibition of most of the PAF effect at the concentraion showed the maximal effect on either LPS- or MDP-stimulated AM. Above results in this study strongly suggest a specific mechanism for PAF action on IL-1 activity mediated by a putative cell membrane receptor. Inhibitory effect of PAF specific antagonists such as BN 52021, WEB 2086, and CV 3988 was also shown in PAF-induced TNF production by rat alveolar macrophages(Dubois et al, 1989). Specific receptors for PAF have been identified in smooth muscle cells (Hwang et al, 1983), neutrophils(O'Flaherty et al, 1986), macrophages(Valone, 1988), mononuclear leukocytes(Ng & Wong, 1988), eosinophils(Ukena et al, 1989), and kupffer cells(Chao et al, 1989). In addition, successful cloning of the PAF receptor gene from guinea pig lung and several types of cells including human leukocytes(Nakamura et al, 1991) and granulocytes(Kunz et al, 1992) have been characterized, allowing further detailed study of the PAF receptor. Following the activation of specific PAF receptors, diverse biochemical effects are elicited, including activaition of phospholipases C and A_2 leading to hydrolysis of phosphoinosite and release of arachidonic acid, respectively, an increased intracellular calcium cencentration, activation of protein kinase C, protein tyrosine phosphorylation, and proto-oncogene expression(Chao & Olson, 1993). PAF receptor-linked intracellular mechanisms associted with the potentiative effect of PAF on production of cytokines such as IL-1 and TNF by AM, however, have not yet been clearly elucidated.

In summary, PAF can potentiate IL-1 activity by rat alveolar macrophages stimulated bacterial compounds such as MDP and LPS, and toxic respirable dust, silica. This action of PAF on AM is strongly proposed to be mediated via interaction with speccific membrane binding sites. The net results should provide additional information and mechanism in which PAF may play a potent role in lung inflammation and damage associated with microbe and toxic respirable dust exposures.

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