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Platelet-Activating Factor Potentiates the Activity of Respiratory Burst and Interleukin-1 in Rat Alveolar Macrophages

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=ABSTRACT=

The objective of the present study was to test the effect of platelet-activating factor (PAF) on rat alveolar macrophages. PAF alone did not stimulate superoxide secretion from alveolar macrophages. However, PAF (10^{-5} M) significantly enhanced phagocytic activator zymosan-induced superoxide secretion from alveolar macrophages. This enhancement of PAF plus zymosan was 30% above the sum of the separate effects of PAF and zymosan. Similarly, PAF (1.3×10^{-5} M) was not a direct stimulant of alveolar macrophages, as it had no stimulatory effect on chemiluminescence generation, but potentiated zymosan-induced activation of chemiluminescence, i.e., 162% above the separate effects of each stimulant. PAF ($10^{-16}\sim10^{-6}$ M) also failed to stimulate IL-1 production from alveolar macrophages. In contrast, when both PAF (10^{-10} M) and lipopolysaccharide(LPS) (1 μg/ml) were added together at the initiation of the culture, IL-1 production was significantly increased indicating the potentiative effects of PAF on IL-1 production by alveolar macrophages. Collectively, these data suggest that PAF alone does not activate the release of bioactive products from alveolar macrophages. However, PAF appears to act as a priming mediator that potentiates stimuli-induced macrophage activity. These novel actions of PAF prove its role as a potent mediator of inflammatory and immune responses in the lung.

Key Words: Plapelet-activating factor, Respiratory burst, Interleukin-1, Rat alveolar macrophages

INTRODUCTION

PAF(1-O-alkyl-2-O-acetyl-sn-glycerol-3-phosphorylcholine) was first described as a mediator released from IgE-sensitized basophils (Benveniste et al, 1972). It was named for its property to induce the rapid aggregation of platelets and secretion of their granule constituents. Recently, PAF has emerged as a potential mediator in pulmonary inflammation, fibrosis, edema, emphysema, asthma and hypersensitivity pneumonitis since PAF is produced by various cell types including alveolar macrophages, neutrophils and basophils after stimulation with zymosan, endotoxin, chemotactic agents, calcium ionophore, and phorbol esters(Arnous & Duval, 1980; Rylander & Beijer, 1987; Betz & Henson, 1980; Benveniste et al, 1972). Released PAF exhibited a variety of biological effects on the lungs. It causes enhancement of capillary permeability (Palade et al, 1979; Northovers, 1993) and constriction of the smooth muscle in the lung(Denjean et al, 1983; Jancar et al, 1987). Furthermore, PAF stimu-

lates neutrophils to induce the release of bioactive products such as lysosomal enzymes(Shaw et al, 1981), reactive oxygen radicals(Van Dyke & Castranova, 1987) and arachidonate metabolites(Levi et al, 1982; Chen et al, 1994). The responses of alveolar macrophages, which play an important role in the first defensive mechanisms as pulmonary phagocytic cells, have been the subject of only a few studies.

The present study has now addressed the question of whether PAF activates rat alveolar macrophages, as reflected by induction of respiratory burst and cytokine release.

MATERIALS AND METHODS

Chemical reagents

PAF (Sigma Co., MD, USA) was dissolved in ethanol and suspended in phosphate-buffered solution (PBS) containing bovine serum albumin (0.25 %). Zymosan (Sigma Co., MD, USA) was prepared in HEPES-buffered medium, boiled, and sonicated to disperse the zymosan particles. LPS from Escherichia coli (Sigma Co., MD, USA) was dissolved in distilled water.

Cellular isolation

Alveolar macrophages were obtained from pathogen-free male Sprague-Dawley rats (250~300 g) (Sweeney et al, 1981). Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (200 mg/kg body weight). The trachea was then cannulated and the lungs lavaged 10 times with 8 ml aliquots of Ca^{++}, Mg^{++} free Hank's 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. Cells were counted using an electronic cell counter, and suspended in HEPES-buffered medium for the measurement of respiratory burst by alveolar macrophages or 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 for the measurement of IL-1 production.

Measurement of superoxide anion secretion

Superoxide secretion was monitored spectrophotometrically at 550 nm by measuring the reduction of cytochrome C (Sweeney et al, 1981). Alveolar macrophages (4×10^{-6}) were preincubated for 10 min at 37℃ in 6 ml of HEPES-buffered medium (145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM HEPES and 5.5 mM glucose, pH=7.4). After preincubation, PAF (10^{-6}-2×10^{-5} M) in the presence or absence of zymosan (0.12 mg/ml) was added to cell suspension. At zero time and 30 min after incubation, 3 ml samples were taken and the cells were immediately separated from the supernates by centrifugation at 2,000 g for 1 min. The optical densities of the supernates were then measured at 550 nm. Superoxide secretion has been shown to be proportional to the difference between the optical densities at 30 and zero time (Drath et al, 1976). The optical densities were converted to nmoles/10^6 cells.

Measurement of chemiluminesence generation

Chemiluminescence generated from alveolar macrophages was measured using a Berthold 9505 luminometer as described by Castranova et al (1989). Alveolar macrophages (3×10^6) were preincubated in 500 μl of HEPES-buffered medium containing luminol (10^{-5} M) in the presence or absence of PAF (1.3×10^{-5} M) and zymosan was added at zero time. Chemiluminesence was analyzed by an on-line computer to obtain total generation for 10 min.

Measurement of IL-1 production

Aliquots of 1 ml containing 10^6 alveolar macrophages were added to 24 well plates and incubated for 2 hr at 37℃ in a humidified atmosphere of 5% CO_2. The nonadherent cells were then removed with

two 1 ml washes of the RPMI media. The adherent cells were further incubated in 1 ml of the RPMI media containing PAF (10^{-16}-10^{-6} M) in the presence or absence of lipopolysaccharide from Escherichia coli (1 μg/ml). After incubating for 20 h, the supernates were collected, filtered through a 0.22 μm sarstedt filter and frozen at -70℃ until they were assayed. Rat alveolar macrophage supernatants 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 streptomysin, 100 units/ml nystatin, 10% heat-inactivated fetal calf serum, and 2×10^5 M mercaptoethanol. Cells were counted using an hemocytometer and adjusted to a concentration of 10×10^6 cells/ml. An aliquot of 100 μl of the macrophage-conditioned supernates was placed in each well. Appropriate samples contained medium, PAF and/or LPS. Cultures were incubated for 66 h in humidified CO_2 at 37℃, pulsed with [^{3}H] thymidine (1.0 μCi/well, activity: 2.0 Ci/mmol, Dupont NEN Products, Boston, MA), and harvested at 72 h 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 like 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 $p<0.05$.

RESULTS

In the present investigation, to determine first, whether PAF directly activates alveolar macrophages and second, whether the responses of these cells to a second stimulant were augmented by PAF, respiratory bursts such as superoxide anion secretion and chemiluminesence genearation, and IL-1 activity in alveolar macrophages have been measured in the presence of PAF and /or a secondary stimulant.

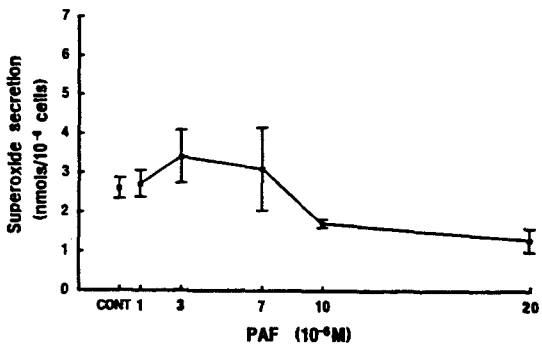

Fig. 1. Effect of PAF on superoxide anion secretion from rat alveolar macrophages. Superoxide secretion was monitored by measuring cytochrome C reduction. Cells were preincubated for 10 min at 37℃ and exposed to PAF (10^{-6}-2×10^{-5} M) just prior to measurement of superoxide anion secretion. Data represent means ± SEM of six experiments.

Superoxide secretion from rat alveolar macrophages in the presence of PAF alone was not significantly increased for concentrations ranging from 10^{-6} to 2×10^{-5} M above the resting level of 2.56 ± 0.24 nmoles/10^6 cells. (Fig. 1). However, phagocytic activator zymosan (0.12 mg/ml) caused a 19.4 fold increase; and the concomitant addition of PAF (10^{-5} M) and zymosan (0.12 mg/ml) to alveolar macrophage further enhanced superoxide secretion (Fig. 2). This enhancement of PAF plus zymosan was 30% above the sum of the separate effects of PAF and zymosan. Compared to the active form of PAF, the biologically inactive PAF precursor, lyso-PAF failed to induce any significant superoxide stimulation with or without zymosan.

Similar results were obtained in assays of chemiluminescence (Fig. 3). PAF (1.3×10^{-5} M) did not

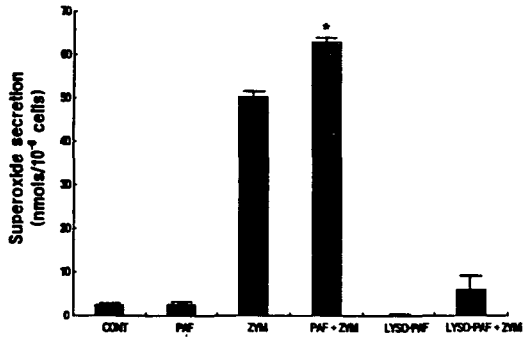

Fig. 2. Effects of PAF and Lyso-PAF on superoxide anion secretion from rat alveolar macrophages. Superoxide secretion was monitored by measuring cytochrome C reduction. Cells were preincubated at 37℃ for 10 min and added to PAF (10^{-5} M) or Lyso-PAF (10^{-5} M) in the absence or presence of zymosan (0.12 mg/ml) just prior to measurement of superoxide anion secretion. Data represent means±SEM of five experiments. Asterisk indicates a significant increase above the sum of the separate effects of each stimulant at $p<0.05$.

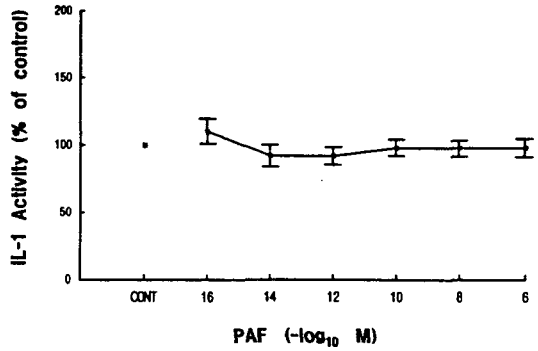

Fig. 4. Effects of PAF on IL-1 production by rat alveolar macrophages. Cells were cultured in the presence of PAF (10^{-16}–10^{-6} M). Cell-free supernatants were collected after 20 h, and IL-1 production was measured in the thymidine incorporation assay using mouse thymocytes. Data are expressed as percentage over control levels obtained from nonstimulated cells, and represent means±SEM of five experiments.

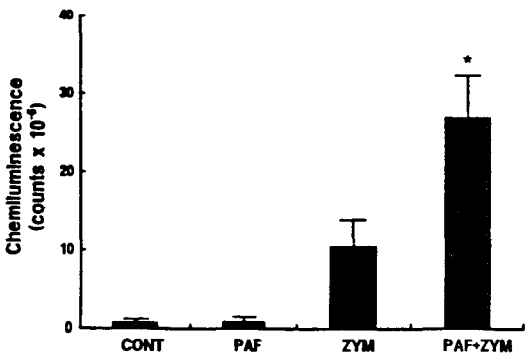

Fig. 3. Effects of PAF on chemiluminescence generated from rat alveolar macrophages. Chemiluminescence was measured in the presence of luminol (10^{-5} M). Cells were preincubated with PAF (1.3×10^{-5} M) or buffer 15 min before the addition of zymosan (2 mg/ml) and measurement of chemiluminescence. Data represent means± SEM of three experiments. Asterisk indicates a significant increase above the sum of the separate effects of each stimulant at $p<0.05$.

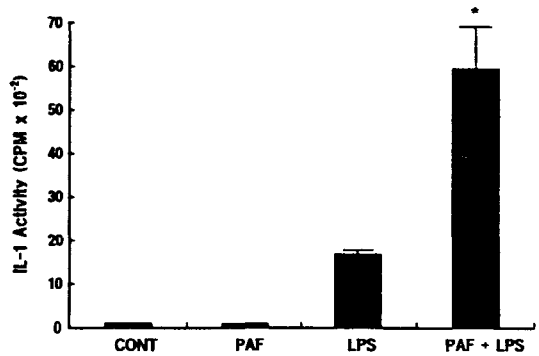

Fig. 5. Effects of PAF on IL-1 production by rat alveolar macrophages. Cells were cultured with PAF (10^{-10} M) in the absence or presence of LPS (1 μg/ml). After 20 h incubation, supernatants were collected and IL-1 production measured by the thymidine incorporation assay using mouse thymocyte. Data represent means± SEM of four experiments. Asterisk indicates a significant increase above the sum of the separate effects of each stimulant at $p<0.05$.

significantly induce the generation of chemiluminescence by alveolar macrophages, i.e., 1.1 fold above the resting level of 816,635 counts for 10 min. In contrast, preincubation of alveolar macrophages with

PAF for 15 min prior to the addition of zymosan(2 mg/ml) increased chemiluminescent reaction of macrophages to 2.5 fold above that caused by zymosan alone. This potentiation of 162% above the sum of the separate effects of each stimulant was significant ($p<0.05$.)

When graded concentrations (10^{-16}-10^{-6} M) of PAF alone were added to the macrophages culture, no significant effect on IL-1 production was observed (Fig. 4). LPS (1μg/ml) exhibited 15.5 fold increase (Fig 5). When both PAF (10^{-10} M) and LPS (1 μg/ml) were added together at the initiation of the culture, IL-1 production was elevated by 3.5 fold above the LPS effect. In this case, potentiated activation was 268% above the sum of the separate effects of PAF and LPS.

DISCUSSION

Alveolar macrophages play a pivotal role in the lung defense system, and they set up the main link between primary inflammatory events and the ensuing immune response. Several macrophage functions are mediated through their ability to release potent 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), reactive oxygen radicals, complement C_{5a} (Reynolds, 1983), and various kinds of cytokines (King et al, 1989; Kelly, 1980).

In the present study, it was determined whether PAF activated alveolar macrophages resulting in the secretion of reactive oxygen radicals and IL-1 production.

The results indicate that PAF does not directly stimulate superoxide secretion and chemiluminescence generation from alveolar macrophages. However, PAF markedly potentiates zymosan-induced respiratory burst activity by alveolar macrophages. In comparison to the present results from alveolar macrophages, it has been demonstrated that PAF alone directly stimulates respiratory burst activity of human polymorphonuclear leukocytes (PMN) as well as primed formyl-methionyl-leucyl-phenylalanine (FMLP)-or phorbol myristate acetate (PMA)-induced PMN activation (Worthen et al, 1988; Vercellotti et al, 1988). It was suspected whether such a disagreement with results may be due to species differences, i.e., rat macrophages compared to human neutrophils. To test this possibility, human alveolar macrophages from a healthy volunteer were harvested by pulmonary lavage under the bronchoscopic guide and the effect of PAF was tested. As with rat alveolar macrophages, PAF (10^{-5} M) did not directly stimulate superoxide secretion from human alveolar macrophages (data not shown). Thus, the disagreement in the results may be caused by different responses between alveolar macrophages and PMN to PAF rather than species differences. This conclusion is supported by the results of Kato et al (1993) where I.V. administrated PAF did not enhance O_2^- generation by guinea-pig alveolar macrophages, but there was a significant increase in a PMA- or FMLP-stimulated O_2^- generation in a dose-dependent manner.

The mechanism by which PAF modulates the respiratory burst responses of alveolar macrophages remains unclear. Recently, Pendino et al (1993) reported that alveolar macrophages from rats expressed low numbers of PAF receptors and PAF failed to induce intracellular calcium mobilization in the cells. In contrast, O_3 exposure in vivo up-regulated PAF receptors on rat alveolar macrophages and induced a rapid and transient rise in intracellular calcium. Their study suggests that PAF receptors on resting alveolar macrophages are inactive. However, oxidative stimulants such as zymosan and O_3 may be required for the number and activity of PAF receptors to be elevated eliciting its functional reponses.

With regard to its effects on the respiratory burst activity of alveolar macrophages, PAF alone fails to stimulate IL-1 production from alveolar macrophages, but PAF potentiates LPS-stimulated response

of these cells. This finding has also been observed in other type of cytokine production, such as tumor necrosis factor and IL-6 from rat alveolar macrophages (Dubois et al, 1989; Thiverge et al, 1992). Furthermore, it has been suggested that the action of PAF on cytokine production can be mediated by the generation of endogenous lipoxygenase metabolites, i.e., leukotriene B_4. These results may correlate with the finding of Glaser et al (1990), who showed that both PAF and LPS were needed to trigger phospholipase A_2 synthesis and activity in the macrophage-like cell line, $P388D_1$. Therefore, the direct measurement of lipoxygenase and cyclooxygenase metabolites in the supernates of rat alveolar macrophages stimulated with PAF in the presence or in the absence of LPS, as well as the study on effects of enzyme inhibitors of arachidonate metabolic pathway on the IL-1 production, might provide information on the mechanism of action of PAF.

In conclusion, PAF alone does not activate the release of bioactive products from alveolar macrophages. However, PAF appears to act as a priming mediator that potentiates stimuli-induced macrophage activity. Clearly, further investigation is needed to fully understand the modulating action of PAF and the mechanisms by which it may regulate the inflammatory and immune responses.

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