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Effects of Platelet-Activating Factor on Tumor Necrosis Factor-α Production by Muramyl Dipeptide- or Silica-Stimulated Alveolar Macrophages

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=ABSTRACT=

Platelet-activating factor(PAF) is a phospholipid mediator of pulmonary inflammation, and immunologic reaction. In this study, the role of PAF on tumor necrosis factor(TNF-α) production by rat alveolar macrophages(AM) was examined. When PAF (10^{-12}~10^{-6} M) alone was added to AM culture, TNF-α production was not significantly increased above the resting level. In contrast, the combined addition of PAF (10^{-6} M) and muramyl dipeptide(MDP) (1.0 μg/ml) to AM cultures markedly enhanced TNF-α production with 8.2 fold increase compared with AM culture in resting state. This potentiative effect was 313% above the sum of the separate effects of PAF and MDP. To characterize MDP effects on TNF-α production, the dose-response of AM cultured with various concentrations of MDP was tested. High level of MDP (10 μg/ml) could not significantly enhance the potentiative effect on TNF-α production compared with AM cultures with low level of MDP (0.1 μg/ml), i.e. 112.5% vs 107.8%, respectively when 10^{-10} M of PAF was simultaneously added to the cell culture. These data support that the potentiation of TNF-α production in AM culture is mediated by PAF rather than MDP. It was also evaluated whether the similar result was obtained in silica, respirable toxic particle-treated AM culture. TNF-α production was also significantly enhanced in the PAF (10^{-6} M) and silica (50 μg/ml)-added cell cultures with 4.7 fold above the value of silica alone-stimulated cells. These results indicate that PAF can potentiate TNF-α production by MDP- or silica- stimulated AM and suggest that PAF may play a potent role in lung inflammation and disease associated with microbe and occupational dust exposures.

Key Words: PAF, TNF-α, Alveolar macrophages, MDP, Silica

INTRODUCTION

TNF-α is a proinflammatory cytokine produced primarily by cells of macrophages/monocyte lineage in response to bacterial products (Beutler & Cerami, 1986; Le & Vilcek, 1987) and toxic particles (Rosenthal et al, 1989; Driscoll et al, 1989). In addition to the well-known cytotoxic and antitumor properties of TNF-α (Carswell, et al, 1975), this cytokine was

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shown to exert a critical role in the development of acute pulmonary failure and injury in states of sepsis (Tracey et al, 1988).

This is presumably a direct result of its known effects on endothelial cells and granulocytes, as well as its induction of other mediators such as interleukin-1(IL-1), prostaglandins (Bachwich et al, 1986), and platelet-activating factor (PAF)(Camussi et al, 1987). However, limited data are available concerning the implication of endogenous inflammatory products in TNF regulation. Recently, the involvement of potent lipid mediators in the modulation of macrophage functions has emerged. PAF (1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphocholine), a lipid meadiator, was originally described as a platelet-aggregating mediator released from Ig-E sensitized basophils (Benveniste et al, 1972). PAF release has been demonstrated from basophils, neutrophils, monocytes and alveolar macrophages after stimulation with calcium ionophore, phorbol esters, chemotactic agents, and zymosan particles (Benveniste et al, 1972; Betz & Henson, 1980; Arnoux & Duval, 1980). PAF has a number of pulmonary effects. PAF induces isometric contraction of bronchial smooth muscle (Stimler & O'Flaherty, 1983; Cuss et al, 1986) and an immediate increase in microvascular leakage throughout the respiratory tract in the guinea-pig (Palade et al, 1979; Northover & Northover, 1993). PAF is a chemoattractant for neutrophils and can activate chemiluminescence in these phagocytes (Czarnetzki & Benveniste, 1981; Humphrey & Hanahan, 1982; Van & Castranova, 1987). In macrophages, PAF stimulates chemotaxis, oxygen burst, and oncogen (Yasaka et al, 1982; Hartung, 1983; Huang et al, 1988; Pignol et al, 1987; Ho et al, 1987). Furthermore, PAF is probably best considered as a mediator acting among a network of mediators involved in chronic inflammatory processes since interactions of PAF with a range of cytokines and with arachidonic acid products are illustrated (Dubois et al, 1989)

In the present study, it was examined whether PAF plays a role in modulation of TNF-α production by alveolar macrophages. Evidence was obtained that PAF alone had minor effect on TNF-α production by alveolar macrophages cultured. However, in association with a second stimulant such as MDP and silica, PAF markedly potentiated TNF-α production.

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 %). MDP was purchased from Calbiochem (CA, USA) and dissolved in saline. Crystalline silica (particle size $<$ 10 μm, Sigma Co., MD, USA) was sterilized by heating at 160 ℃ for 90 min in a dry oven and dispersed in RPMI media with supplements just before addition to culture plates.

Alveolar macrophage cultures and supernate preparation

Alveolar macrophages were obtained from male Sprague-Dawley 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 ㎖ 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, counted using an hemocytometer, 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. 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 ㎖ washes of the RPMI media. The adherent cells were

further incubated in 1 ml of the RPMI media containing PAF (10^{-12}-10^{-6} M) in the presence or absence of MDP (1.0 μg/ml) or silica (50 μg/ml). After incubating for 20～24 h, the supernates were collected, filtered through a 0.22 μm Sarstedt filter and frozen at -70° C until they were assayed.

Enzyme immunoassay for TNF-α

Production of TNF-α was measured in terms of immunoreactive TNF-α using a commercial ELIZER immunoassay kit (Biosourse, CA, USA) and expressed as pg/㎖ using TNF-α standard curves.

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

The objective of the present investigation is to determine if PAF directly stimulate TNF-α production by alveolar macrophages and to determine if PAF potentiates the response of theses cells to a second stimulant.

When adherent alveolar macrophages were cultured with graded concentrations of PAF(10^{-12}～10^{-6}M), producion of TNF-α was not significantly increased above the basal level of 111.04±66.04 pg/ml (Fig.1). MDP (1.0 μg/ml), bacterial cell wall component, caused 2.0 fold increase above the basal TNF-α; the combined addition of PAF (10^{-6} M) and MDP (1.0) μg/ml to alveolar macrophage cultures markedly enhanced TNF-α production with 8.2 μg/ml fold increase in TNF-α compared with the control of resting state(Fig. 2). This potentiation of 313.0% above the sum of the separate effects of PAF (10^{-6} M) and MDP (1.0 μg/ml) was significant($p < 0.01$) (Fig. 1 and Fig. 2). Below 10^{-8} M of PAF, MDP (1.0 μg/ml)-stimulated alveolar macrophages showed tendency of increase in TNF-α production above the

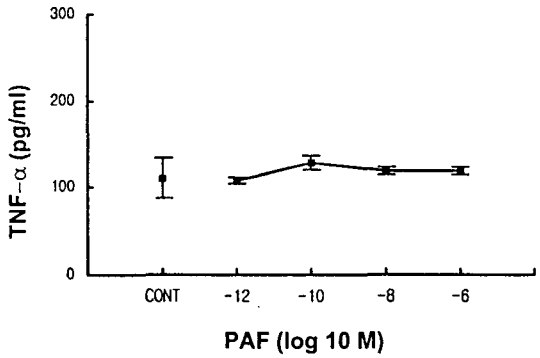

Fig. 1. Effects of PAF on TNF-α production by alveolar macrophages. Adherent cells were cultured in the presence of PAF (10^{-12}～10^{-6} M) and cell- free supernates collected after 20 h. Production of TNF-α was measured in terms of immunoreactive TNF-α using a ELIZER immunoassay (Biosourse, CA, USA) and expressed in pg/ml using TNF-α standard curves. Data represent means±SEM of four experiments.

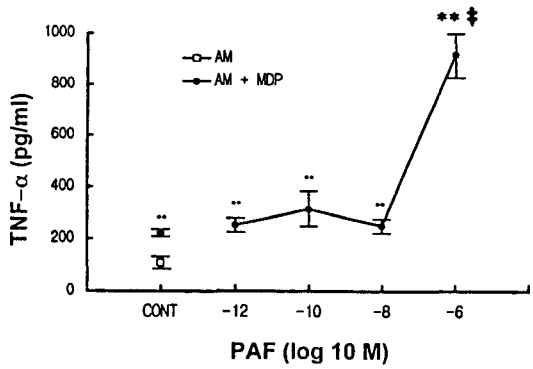

*Fig. 2. Effect of PAF on TNF-α production by alveolar macrophages stimulated by MDP. Adherent cells were cultured with PAF (10^{-12}～10^{-6} M) and MDP (1.0 μg/㎖) and cell-free supernates collected after 20 h. Production of TNF-α was measured in terms of immunoreactive TNF-α using a ELIZER immunoassay (Biosourse, CA, USA) and expressed in pg/ml using TNF-α standard curves. Data represent means±SEM of four experiments. * $p < 0.05$, ** $p < 0.01$ for MDP- or MDP- + PAF- stimulated cells compared with unstimulated cells. ‡ $p < 0.01$ for MDP- + PAF-stimulated cells compared with MDP-stimulated cells.*

Table 1. Effect of various concentrations of MDP on TNF-α production in alveolar macrophage cultures with or without PAF

Stimuli: MDP(μg/mℓ)	Stimuli: PAF(M)	TNF-α production(pg/mℓ)
None	None	110.28±23.41
0.1	0	155.01±20.31
1.0	0	260.83±33.01**
10.0	0	547.08±35.32**
0.1	10^{-10}	167.08±13.27
1.0	10^{-10}	305.00±27.35**
10.0	10^{-10}	615.42±41.01**

Data represent means±SEM of three experiments. ** p <0.01 for MDP- or MDP- + PAF-stimulated cells compared with unstimulated cells.

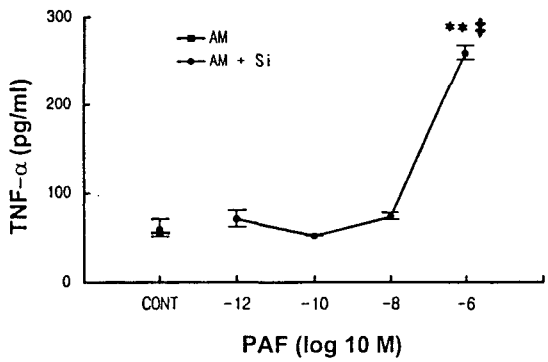

*Fig. 3. Effect of PAF on TNF-α production by alveolar macrophages stimulated by silica. Adherent cells were cultured with PAF (10^{-12}~10^{-6} M) and silica (50 μg/mℓ) and cell-free supernates collected after 20 h. Production of TNF-α was measured in terms of immunoreactive TNF-α using a ELIZER immunoassay (Biosourse, CA, USA) and expressed in pg/ml using TNF-α standard curves. Data represent means±SEM of four experiments. ** p<0.01 for silica- + PAF- stimulated cells compared with unstimulated cells. ‡ p<0.01 for silica- + PAF-stimulated cells compared with silica-stimulated cells.*

value of cell cultured with MDP (1.0 μg/ml) alone.

To characterize effects of MDP on TNF-α production, the different doses of MDP (0.1~10 μg/ml) were added to alveolar macrophages cultured with or without PAF (10^{-10} M). MDP stimulated TNF-α production in a dose- dependent fashion (Table 1). However, high level of MDP (10 μg/ml) could not enhance the potentiative effect on TNF-α production compared with alveolar macrophage cultures with low level of MDP (0.1 μg/ml), i.e., 112.5 % vs 107.8 %, respectively when 10^{-10} M of PAF was simultaneously added to the cell culture. This result suggests that the potentiation in TNF-α production is mediated by PAF rather than MDP.

To evaluate whether other macrophage activator could bring the similar result, silica (50 μg/ml), respirable toxic particle, was treated to alveolar macrophage cultures with or without graded concentrations of PAF (10^{-12}~10^{-6} M). As shown in Fig. 3, PAF also exhibited potentiative effect on the TNF-α production with the peak response at the concentration of 10^{-6} M PAF. The data indicate that silica alone did not increase TNF-α production, but, PAF (10^{-6} M) and silica together increased 4.7 fold TNF-α production over the value of silica-stimulated cells. There were slight variations of the dose-response curve from experiment to experiment, particularly below 10^{-8} M PAF wherese maximal TNF-α production always occurred at 10^{-6} M.

DISCUSSION

In the present study our data provided evidence that (1) PAF alone can not increase TNF-α production by rat alveolar macrophages. (2) However, TNF-α production is potentiated by PAF with a second stimulant such as MDP and silica. (3) The peak response of this effect is always observed at 10^{-6} M PAF.

Failure of PAF alone to stimulate TNF-α in rat alveolar macrophages suggests that a double signal, provided by PAF with MDP or silica was needed to trigger the priming effect. This may correlate with the findings of Glaser et al (Glaser et al, 1990) who

reported that both lipopolysaccharide and PAF were needed to trigger phospholipase A_2 synthesis and activity in the macrophage-like cell line, $P388D_1$

Other laboratories have recently reported modulation of TNF production by PAF in cultures of human monocytes and lymphocytes. Poubelle et al (1991) showed TNF production with a major enhancement at 10^{-8}-10^{-6} M and a second enhancement at 10^{-15}-10^{-14} M. Human large granular lymphocytes also produce augmented amounts of TNF following stimulation with 10^{-14} and 10^{-10} M PAF (Bosse, Turcotte & Rola-Pleszczynski, 1991). Recently, Barthelson et al (1988) showed that the THP-1, human monocytic leukemia cell line, also responded to PAF in a multiphasic dose-response fashion. Our study supports this augmenting effect of PAF on TNF-α production which was demonstrated in rat alveolar macrohpage cultures with MDP or silica. The peak response was always observed at 10^{-6} M PAF with MDP or silica. However, in the range of 10^{-12} to 10^{-8} M the potentiative effect of PAF on MDP- or silica- simultaneously added AM cultures was not observed. The variations of the dose-reponse curve between rat alveolar macrophage (i.e., peak response at 10^{-6} M) in our data and human monocyte culture (i.e., peak response at 10^{-8} M) (Poubelle et al, 1991) may be in part due to species and cell subpopulation heterogeneity which may represent different functional states or maturational levels.

It has also recently reported that PAF regulates other cytokine production in culture of macrophage-monocyte. Salem et al (1990) showed enhanced IL-1 production by LPS- or MDP-stimulated monocytes, at PAF concentrations of 10^{-12}-10^{-8} M or higher than 10^{-9} M respectively. Pignol et al (1987) reported enhanced production of IL-1 by splenic macrophages at 10^{-12} M PAF. Similar responses in IL-1 as well as IL-2 were obtained in spleen cells from rats chronically infused with PAF (Pignol et al, 1990). In addition, Thiverge and Rola-Pleszczynski (1992) have reported enhanced production of Il-6 by 10^{-10}-10^{-8} M PAF with peak effect at 10^{-10} M. This ability of PAF to induce the production of various cytokines may be one of the several possible mechanisms of microbes-, immune complex- and toxic particle- induced inflammatory and immune reactions. Not only does PAF stimulate synthesis of cytokines and itself (Tessner et al, 1989; Vaone, 1991), but cytokines can induce the synthesis of more PAF (Valone & Epstein, 1988; Bussolino et al, 1986), thereby completing a cycle of positive feedback loops. These loops could be important for the amplification of the immune response.

In conclusion, the present data indicate that PAF can potentiate TNF-α production by MDP- or silica-stimulated alveolar nacrophages and provide additional information in which PAF may play a potent role in lung inflammation and disease associated with microbe and occupational dust exposures.

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