Priming by Platelet-Activating Factor of Endotoxin-Induced Lung Injury and Cardiovascular Shock

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Platelet-activating factor (PAF) is a glycerophospholipid known for its unusual potent vasoactive and proinflammatory activities. The present study examined whether PAF might serve as a priming factor in endotoxin-induced tumor necrosis factor-α (TNFα) synthesis, cardiovascular shock, and lung injury in anesthetized rats. Intravenous infusion of PAF (1 pmol/kg/min for 60 minutes, n = 5) alone or endotoxin (0.1 μg/kg i.v. bolus, n = 5) failed to alter blood pressure, serum TNFα and thromboxane B₂, platelet and leukocyte count, and hematocrit, nor was lung histology, myeloperoxidase activity, and water content changed. In contrast, the combined administration of PAF and endotoxin markedly elevated serum TNFα (1,359 ± 362 pg/ml, n = 5, p < 0.01) and thromboxane B₂ (43 ± 5 pg/100 μl, n = 8, p < 0.01) along with hypotension, hemoconcentration, leukopenia, and thrombocytopenia. Most notably, the combined regimen caused neutrophil aggregation, adhesion, and accumulation into the lung parenchyma along with platelet-fibrin deposits in postcapillary venules, pulmonary edema, and increased lung myeloperoxidase activity. The role of PAF in this process was confirmed by 1) the prevention of the priming effect by pretreatment with the PAF antagonist BN 50739 (n = 5), and 2) the failure of lyso-PAF, the cardinal nonactive PAF-metabolite, to prime for endotoxin-induced production of TNFα (n = 4). These data suggest that PAF could serve as a key mediator in priming for endotoxin-induced tissue injury, especially the typical pulmonary pathophysiology of adult respiratory distress syndrome, a severe pathological outcome of septic shock, burns, and multiple organ injury. (Circulation Research 1991; 69:12–25)

Septic shock is a complex syndrome involving multiple mediators among which platelet-activating factor (PAF) and tumor necrosis factor-α (TNFα) play a major role. Many of the manifestations of endotoxic shock, such as hypotension, hemoconcentration, thrombocytopenia, leukopenia, and death, can be produced by the systemic administration of PAF or TNFα. Also, elevated serum levels of PAF and TNFα were found in lipopoly saccharide (LPS)-injected animals, and increased TNFα activity was detected in septic patients. PAF antagonists or TNFα-neutralizing antibodies each were shown to convey significant protection in animal models of septic shock. Furthermore, enhanced release of PAF from splenic lymphocytes obtained from rats exposed to bacterial peritonitis and the release of TNFα by monocytes and macrophages stimulated by LPS were reported as well.

Thus, it is currently believed that LPS exerts its pathophysiological effects primarily through the production of PAF and TNFα, but the relation between these mediators is poorly understood. Several in vitro studies reported that PAF and TNFα are able to elicit production of each other from cultured macrophages. In addition, TNFα was shown to prime PAF-induced superoxide production by human neutrophils. However, the nature of the interaction between PAF and TNFα in mediating endotoxin effects is virtually unknown. The only in vitro study that attempted to explore such relations demonstrated that pretreatment or posttreatment of isolated murine macrophages with a PAF antagonist inhibited TNFα production in response to endotoxin and that high concentrations of the PAF antagonist added to the macrophage medium simultaneously with endotoxin completely inhibited TNFα.
mRNA expression; however, no direct measurements of PAF were provided in that study. In in vivo studies, pretreatment with the PAF antagonist BN 50739 attenuated the LPS-induced elevation of serum TNFα and decreased TNFα-induced mortality.17 Support of the possible linkage of PAF and TNFα in septic states was provided by Heuer et al,18 who have demonstrated that pretreatment of mice with pharmacological doses of either *Salmonella typhosa* endotoxin or TNFα resulted in a synergistic increase in PAF-induced mortality. However, when administered alone at similar doses, neither TNFα nor endotoxin had any effect on animal survival. In contrast, Myers et al19 observed no beneficial effect of pretreatment or posttreatment with the PAF antagonist BN 52021 on the lethality of combined low-dose endotoxin and recombinant human TNFα, which singly had no effect in mice. However, the priming effect of PAF on TNFα release was challenged by studies showing that TNFα production by blood monocytes exposed to LPS was suppressed if monocytes were first primed by PAF.20

Taken together, these data suggest that PAF/TNFα interactions may play a role in the mediation of endotoxin-induced pathophysiological responses. The purpose of the present study was to investigate this hypothesis in vivo and to examine a possible priming relation of PAF on LPS-induced TNFα production, cardiovascular shock, and lung injury in vivo.

**Materials and Methods**

**Materials**

PAF (1-α-phosphatidylcholine, β-acetyl-γ-O-hexadecyl) was purchased from Sigma Chemical Co., St. Louis, and was dissolved in 0.25% essentially fatty acid–free bovine serum albumin (Sigma) in 0.9% NaCl. The stock solution was diluted in vehicle as needed, for infusion for 60 minutes in a total volume of 2 ml. *Escherichia coli* 0111:B4 (Sigma) was used as a fresh solution in 0.9% NaCl.

BN 50739, a potent, selective, and long-acting PAF antagonist,21 kindly provided by Dr. P. Braquet (Institut Henri Beaufour, Paris), was solubilized in 64% dimethyl sulfoxide in 0.9% NaCl to reach a concentration of 5 mg/ml.

Lyso-PAF (1-α-lyso phosphatidylcholine, γ-O-hexadecyl, Sigma) was prepared in the same bovine serum albumin/NaCl vehicle as PAF and was administered at 1 pmol/kg/min for 60 minutes in a total volume of 2 ml.

**Animals**

Male Sprague-Dawley rats weighing 303–356 g (Taconic Farms, Germantown, N.Y.) were studied. Until surgery, all animals were housed in groups of two in standard cages and provided with food and water ad libitum in a temperature-controlled room (22°C) on a 12-hour dark/light cycle.

**Primary Experimental Protocols**

TNFα, thromboxane B2, hematocrit, and blood pressure responses to PAF (protocol 1). After anesthesia with pentobarbital (30 mg/kg i.p.), catheters (PE-50) were introduced into the femoral vein for drug infusion and into both femoral arteries for blood pressure measurements and blood sampling. Basal mean arterial pressure (MAP) was recorded, and a blood sample (0.5 ml, exchanged with an equivalent of 0.9% NaCl) was collected for TNFα and thromboxane B2 (TXB2) assay and for hematocrit determination. PAF at 1 (n=5), 10 (n=8), or 100 pmol/kg/min (n=7) or PAF vehicle (control, n=5) was infused at 2 ml/hr for 60 minutes. MAP was continuously monitored for 4 hours, and blood samples (0.5 ml, exchanged with an equivalent of 0.9% NaCl) for TNFα, TXB2, and hematocrit determinations were collected at 30, 60, 120, and 240 minutes after the beginning of infusion. An additional blood sample (0.3 ml, exchanged with an equivalent of 0.9% NaCl) for TXB2 assay was collected at 10 minutes.

TNFα, TXB2, hematocrit, and MAP responses to LPS (protocol 2). The former protocol was repeated with intravenous bolus injection of endotoxin at 0.1 μg/kg replacing the PAF infusion.

TNFα, TXB2, hematocrit, and MAP responses to the combined administration of PAF and LPS (protocol 3). Protocol 2 was repeated using intravenous bolus injection of endotoxin at 0.1 μg/kg, followed by intravenous infusion of PAF at 1 (n=5), 10 (n=5), or 100 pmol/kg/min (n=9) at a rate of 2 ml/min for 60 minutes.

**Additional Protocols**

Because preliminary studies indicated a priming effect of PAF (1 pmol/kg/min) on LPS-induced TNFα release, additional protocols were used to address the following questions:

1) Does the priming result from the action of PAF or is it an effect of lyso-PAF, a key metabolite of the action of plasma acetyldihydrolases on PAF? To address this issue, serum TNFα was determined in a separate group of rats (n=4) before and 30, 60, 120, and 240 minutes after the infusion of lyso-PAF (1 pmol/kg/min for 60 minutes). An additional group (n=4) was used to determine serum TNFα response to the bolus injection of LPS (0.1 μg/kg) followed immediately by the infusion of lyso-PAF (1 pmol/kg/min for 2 minutes, protocol 4); this dose of lyso-PAF was chosen based on the estimate of complete conversion of PAF to lyso-PAF. To further confirm the specificity of PAF in the priming process and to test the possibility that the priming effect of PAF is receptor-mediated, a separate group of rats (n=5) were pretreated (30 minutes) with the PAF antagonist BN 50739 (10 mg/kg i.p.), a new, highly specific and potent PAF-receptor antagonist,21 and protocol 3 was repeated as protocol 5.

2) Is it PAF that primes TNFα response to LPS or is it LPS that primes TNFα response to PAF? Since rat platelets and leukocytes are not sensitive to PAF...
due to lack of PAF receptors, priming effect on platelet and leukocyte count will indicate that PAF primes TNFα response to LPS and not vice versa. Therefore, protocols 1, 2, and 3 were repeated \((n=5)\) with PAF at 1 pmol/kg/min and LPS at 0.1 \(\mu g/kg\), and blood was sampled for platelet and leukocyte count only (protocol 6). In addition, to investigate whether it is LPS that primes for PAF responses, LPS was injected as an intravenous bolus 60 minutes (group I, Figure 1A, \(n=5\)) or 15 minutes (group II, Figure 1A, \(n=5\)) before PAF infusion (1 pmol/kg/min i.v. for 60 minutes) (protocol 7). Blood pressure was monitored for 4 hours, and blood samples for hematocrit, white blood cell and platelet counts, and \(\text{TXB}_2\) and TNFα assays were collected at 0.5, 1, 2, and 4 hours. At the end of the 4-hour observation period both lungs were removed; the right one was taken for myeloperoxidase (MPO) assay, and the left one was used to determine pulmonary water content.

3) Are specific temporal relations of PAF and LPS involved in the interaction that leads to the priming response? To answer this question two sets of experiments were conducted. In the first set, PAF (1 pmol/kg/min i.v.) was infused for 60 minutes, and LPS (0.1 \(\mu g/kg\)) was injected as an intravenous bolus at the beginning of PAF infusion (group III, Figure 1A, \(n=5\)), at the end of PAF infusion (group IV, Figure 1A, \(n=5\)), or 120 minutes later (group V, Figure 1A, \(n=5\)). Blood samples for TNFα assay were collected at 0.5, 1, 2, and 4 hours (protocol 8). In the second set, LPS was injected as a bolus (0.1 \(\mu g/kg\)) followed by PAF infusion (1 pmol/kg/min) over 5 minutes (group III, Figure 1B, \(n=5\)), 15 minutes (group II, Figure 1B, \(n=5\)), or 60 minutes (group I, Figure 1B, \(n=5\)). Blood samples for TNFα assay were collected at 0.5, 1, 2, and 4 hours (protocol 9).

4) Is it possible that PAF serves as an accelerator for LPS-induced effects rather than a priming mediator for LPS pathophysiological actions? To exclude this possibility it should be demonstrated that LPS (0.1 \(\mu g/kg\)) would not by itself produce the changes elicited by the combined administration of PAF and LPS even at later periods of time. Therefore, an endotoxin bolus was given to a separate group of chronically instrumented rats \((n=5)\), and MAP, hematocrit, leukocytes, platelets, and serum TNFα were determined at 8, 12, 24, and 48 hours (protocol 10).

5) Is PAF priming effect related to LPS in a dose-dependent manner? To address this question, protocols 1, 2, and 3 were repeated using LPS at 0.01 \(\mu g/kg\) (protocol 11, \(n=5\)). MAP, hematocrit, and TNFα levels were determined.

**Pulmonary histopathology.** The latter protocol was repeated \((n=5)\) with the right lung removed 4 hours after treatment and fixed while inflated with phosphate-buffered formalin (pH 7.2). The lobes were divided, and one lobe was processed for paraffin sections and stained with hematoxylin and eosin using routine methods. A second lobe was further fixed in 3% glutaraldehyde in 0.1 M phosphate buffer and processed for scanning electron microscopy. These lobes were dehydrated in ethanol incubated in hexamethyldisilazane and dried under vacuum. After drying, each lobe was sliced into four pieces, mounted on a specimen stub, and sputter-coated with gold. The lobes were examined with a Hitachi S-570 scanning electron microscope operating at 20 kV at a working distance of less than 5 mm.

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**Figure 1.** Schematic presentation of the experimental protocols designed to evaluate the temporal relation in the platelet-activating factor (PAF) and lipopolysaccharide (LPS) interaction, which leads to the priming response. Panel A: PAF (1 pmol/kg/min) infused for 60 minutes and LPS (0.1 \(\mu g/kg\)) injected as a bolus at 60 (group I) or 15 minutes before (group II), the beginning (group III), the end (group IV), or 120 minutes after the end of PAF infusion (group V). Panel B: LPS injected as a bolus at time point 0 and PAF infused for 60 (group I), 15 (group II), or 5 minutes (group III). G, group.
Survival. Survival was monitored for 48 hours in a separate group of rats given LPS (0.1 μg/kg, n=8), PAF (1 pmol/kg/min, n=8), or LPS with PAF (at similar doses, n=10).

Assays

Plasma levels of TNFα were measured by a “sandwich” enzyme-linked immunosorbent assay (ELISA) for which a hamster monoclonal anti-mouse TNFα (Genzyme, Boston) was used as the capture antibody and a polyclonal rabbit anti-murine TNFα (Genzyme) was used as the detecting antibody. TNFα levels in rat samples were calculated from a standard curve generated with recombinant murine TNFα (Genzyme). TNFα levels determined by ELISA correlated with levels detected by the L-929 bioassay, with 1 unit of activity in the bioassay corresponding to 5 pg TNFα in the ELISA. The ELISA detected levels of TNFα down to 25 pg/ml. TXB2 was determined by radioimmunoassay (sensitivity of 7.8 pg/100 μl) as previously described.

MPO activity in the lung was assayed as described previously based on a modification of Bradley’s method.

Pulmonary water content. At the end of the experimental period, the left lung was removed and immediately weighed (wet weight). Dry weight was determined after the lung was dried at 80°C for 36 hours, and the pulmonary water content was calculated by subtracting the lung dry weight from the lung wet weight.

Data Analysis

Data in text and figures are mean±SEM for the indicated number of animals. One-way analysis of variance followed by Student-Newman-Keuls test was used for statistical analysis. Because of the incomplete measurement scale of the TXB2 and TNFα assays (minimal detection level of 7.8 pg/100 μl and 25 pg/ml, respectively), the Kruskal-Wallis test was used for TXB2 and TNFα comparisons. A value of p<0.05 was considered significant for both tests.

Results

Hemodynamic, Hematologic, and Plasma TXB2 Responses to PAF and LPS

Basal MAP (104±9 mm Hg), platelet (810±74×10^5/μl) and leukocyte count (8,090±680/μl), and hematocrit (46.3±0.9%) did not differ among the groups and are in agreement with the normal values of this species of rats. Basal TXB2 levels in all groups were below the minimal detecting level of the assay that was used (7.8 pg/100 μl). This is in agreement with previous studies that used radioimmunoassay.

PAF at 100 pmol/kg/min infused at a rate of 2 ml/hr for 60 minutes induced hypotension (Figure 2), hemoconcentration (Figure 3A), and marked elevation of plasma TXB2 (Figure 4). PAF at 1 or 10 pmol/kg/min; PAF vehicle, administered in a similar fashion; or endotoxin (0.1 μg/kg) did not affect these variables (Figures 2, 3A, 4). Also, PAF at 1 pmol/kg/min had no effect on leukocyte and platelet count. In contrast, the bolus injection of LPS (0.1 μg/kg) followed by PAF infusion (1 pmol/kg/min over 60 minutes) caused hypotension (p<0.01, Figure 2), hemoconcentration (p<0.05, Figure 3A), leukopenia (p<0.05, Figure 3B), thrombocytopenia (p<0.05, Figure 3C), and elevation of plasma TXB2 (p<0.01, Figure 4). Desensitization to PAF effect on LPS-induced hypotension, hemoconcentration, and elevation of plasma TXB2 developed during the last 30 minutes of PAF infusion, as shown by a decrease in these responses at that time.

LPS at 0.1 μg/kg did not affect MAP (basal, 105±10 mm Hg), hematocrit (basal, 45±1%), or platelet (basal, 792±67×10^3/μl) and leukocyte counts (basal, 8.4±0.7×10^5/μl) at 8, 12, 24 and 48 hours (protocol 10). LPS at 0.01 μg/kg did not affect MAP and hematocrit when injected alone or in combination with PAF infusion (protocol 11, Table 1).

TNFα Response to PAF and LPS

Basal TNFα levels were undetectable at the level of the assay sensitivity of 25 pg/ml, which agrees with previous reports in rats. Palmitate-induced hypotension (protocol 4) did not affect serum TNFα (162±70 pg/ml at 4 hours, p<0.05, Figure 5) without dose dependency. PAF at 1 pmol/kg/min or PAF vehicle (Figure 5), infused for 60 minutes, or bolus injection of endotoxin at 0.1 μg/kg (Figure 6) did not affect serum TNFα level.

PAF at all tested doses amplified serum TNFα response to LPS at 0.1 μg/kg (Figure 6). Priming effect was demonstrated at PAF dose of 1 pmol/kg/min (serum TNFα, 1,359±362 pg/ml; p<0.01, Figure 6C) since at this dose the separate infusion of PAF had no effect on serum TNFα activity. In contrast, PAF at doses of 10 (Figure 6B) or 100 pmol/kg/min (Figure 6A) exhibited a synergistic effect, since its separate infusion at these doses evoked a mild, but significant, TNFα response (Figures 5 and 6).

LPS at 0.1 μg/kg did not affect serum TNFα levels also at 8, 12, 24, and 48 hours (protocol 10); likewise, LPS at 0.01 μg/kg did not elevate serum TNFα concentration when injected alone or in combination with PAF infusion (protocol 11, Table 1).

Specificity of PAF in the Priming Effect

The specificity of PAF in the priming process was established by the following observations: 1) Pretreatment with BN 50739 completely prevented (p<0.01) the elevation of serum TNFα induced by the combined administration of PAF (100 pmol/kg/min) and LPS (0.1 μg/kg, Figure 6A), and the infusion of lyso-PAF with or without bolus injection of LPS had no effect on serum TNFα (Figure 7).

Temporal Relation Between PAF and LPS in the Priming Phenomenon

Variations in LPS timing. The infusion of PAF (1 pmol/kg/min for 60 minutes) immediately after the bolus injection of LPS (0.1 μg/kg) primed for LPS-
induced hypotension (Figure 2), hemoconcentration (Figure 3A), leukopenia (Figure 3B), thrombocytopenia (Figure 3C), elevation of serum TXB2 (Figure 4) and TNFα (Figure 6C, Figure 8A), lung edema (Figure 9), MPO activity (Figure 10), and histological changes (Figures 11–13). LPS bolus administered 15 or 60 minutes before PAF infusion (protocol 7) did not affect MAP (basal, 102±12 mm Hg), hematocrit (basal, 46.7±0.6%), platelet (basal, 786±81×10^3/µl) and leukocyte counts (basal, 7,870±768×10^3/µl), serum TXB2 (undetected basal values) and TNFα levels (undetected basal values), and lung water content (basal, 4.40±0.010 g) and MPO activity (basal, 3.5±1.93 units/g wet lung wt). Also, no priming effect on TNFα production was demonstrated when LPS was injected at the end of PAF infusion or 120 minutes later (protocol 8, Figure 8A).

Variations in PAF infusion. The infusion of PAF (1 pmol/kg/min) for 60 or 15 minutes primed the production of TNFα in response to LPS (0.1 µg/kg) injected at the beginning of PAF infusion (protocol 9, Figure 8B). There was no priming effect when PAF was infused for 5 minutes only.

Lung MPO, Weight, and Water Content

The wet (502±5 mg), dry (93±7 mg), and wet-dry (pulmonary water content, 409±9 mg) lung weight of the control rats did not differ statistically from the respective weights in groups of rats receiving PAF or LPS only (Figure 9). In contrast, the combined administration of PAF and LPS significantly elevated pulmonary wet, dry, and wet-dry weight by 18% (n=5, p<0.01) each; the wet-dry/dry ratio (4.40±0.01), however, remained unchanged.

Control animals had pulmonary MPO activity of 4.99±0.54 units/g wet lung wt. This value was not significantly affected by the separate administration of PAF or LPS (Figure 10), whereas bolus injection of LPS followed immediately by PAF infusion increased MPO activity by 519±43% at 4 hours (p<0.01, Figure 10A) and by 606±48% at 24 hours (p<0.01, Figure 10B).
Lung Histopathology

Light microscopy. Lungs removed from rats 4 or 24 hours after the administration of LPS or PAF alone revealed minor morphological change compared with the saline controls where normal lung histology was found. For example, in rats receiving LPS alone, only a few margined polymorphonuclear leukocytes were seen in the pulmonary vein and venules. In contrast, all lungs taken from rats 4 hours after the administration of LPS and PAF in combination revealed uniformly scattered changes compatible with the early phases of adult respiratory distress syndrome (ARDS; for review, see Reference 32), such as adherence of numerous polymorphonuclear leukocytes to vessel walls and alveolar septa, and interstitial infiltration of polymorphonuclear leukocytes (Figure 11). At 24 hours after LPS and PAF were given in combination, the disease process observed at
**FIGURE 4.** Priming effect of platelet-activating factor (PAF) on lipopolysaccharide (LPS)-induced elevation of plasma thromboxane B₂ (TXB₂). Control, PAF vehicle; a, vs. basal value; b, vs. all other groups. The dashed line represents the minimal detecting level of the assay (7.8 pg/100 μl). The inserted plate illustrates TXB₂ response to PAF infusion. #p<0.01.

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LPS, lipopolysaccharide; MAP, mean arterial pressure; PAF, platelet-activating factor; TNFα, tumor necrosis factor-α; UD, undetected. Sensitivity of TNFα assay is 25 pg/ml.

**FIGURE 5.** Tumor necrosis factor-α (TNFα) response to platelet-activating factor (PAF) infusion. Control, PAF vehicle; a, vs. basal value; b, vs. all groups except PAF at 100 pmol/kg/min; c, vs. all groups except PAF at 10 pmol/kg/min. *p<0.05.
4 hours continued; that is, interstitial polymorphonuclear leukocytes were observed in most alveolar septa (Figure 12). Many of these cells demonstrated degenerative changes, suggesting that they had been resident in the lung interstitium for several hours. In the vasculature, numerous leukocytes were adherent to the endothelial lining of the pulmonary veins and venules. In contrast to the 4-hour interval when the adherent leukocytes were predominantly neutrophils, at 24 hours the adherent cells were predominantly monocytes. Also, at the latter time there was interstitial and perivascular edema. There was no evidence of exudation of cells or fibrin into the alveolar spaces at either the 4-hour or the 24-hour interval. Thus, the disease process is characterized histologically as interstitial pneumonia.

**Scanning electron microscopy.** Scanning electron microscopy confirmed and extended the light microscopic findings. The endothelial surface of pulmonary venules from rats given PAF only was free of adherent leukocytes, platelets, or fibrin (Figure 13A). A few platelets and leukocytes were adherent to the
endothelial surface of pulmonary venules taken from rats injected with LPS only (Figure 13B). In contrast, numerous platelets and leukocytes embedded in fibrin strands were adherent to the endothelium of pulmonary venules from rats receiving PAF and LPS in combination (Figure 13C). These leukocytes were covered with numerous blebs and ruffles (in contrast to the fine microvilli in the other groups), probably the result of shortening and broadening of the microvilli, findings previously reported in activated neutrophils.

The above-listed histopathological changes were noticed uniformly in all lung areas and in all five rats. Interestingly, no evidence of adherent leukocytes, platelets, or fibrin was seen in the pulmonary arteries of any of the treatment groups.

**Discussion**

The key finding of this study is that PAF and endotoxin cooperate to produce TNFα, cardiovascular derangements, and lung injury in vivo, possibly through a specific priming effect of PAF on LPS-responsive cells and organs. Our data suggest that PAF serves as a priming factor rather than an accelerator of LPS-induced pathophysiological effects since, when administered alone, LPS did not affect hemodynamic, hematologic, and biochemical variables for 48 hours after injection. In addition, it is suggested that PAF primes for LPS-induced lung injury, cardiovascular shock, and TNFα and TXB2 production and not vice versa (i.e., LPS primes for
PAF effects) since no responses were observed when LPS was administered before PAF infusion. Also, as far as the hematologic responses are concerned, PAF primes LPS action, since the reduction in circulating leukocytes and platelets, which are devoid of PAF receptors and therefore insensitive to PAF,22 was also subject to such a priming effect. Interestingly, in previous models where PAF and LPS acted synergistically to induce tissue injury, LPS injected before PAF augmented PAF responses. For example, Salzer and McCall34 recently demonstrated that low doses of endotoxin that produce little or no effect in isolated perfused rabbit lungs primed the lungs for injury by normally noninjurious doses of PAF. Also, LPS-primed P388D macrophagelike cells increased their PAF-stimulated prostaglandin E2 production.35

The priming effect demonstrated in the present study is a specific and receptor-mediated effect of PAF because the PAF-receptor antagonist BN 5073921 prevented TNFα production in response to the combined administration of PAF and LPS, and because lyso-PAF, the main metabolite of PAF, failed to prime for LPS-induced production of TNFα.

The priming effect of PAF on LPS-induced TNFα production is complementary to a previous observation that endotoxin or TNFα primed PAF-induced mortality and intestinal motility in mice.18 Also, this priming effect of PAF is in accordance with earlier reports that both PAF and TNFα at very low concentrations can prime neutrophils and monocytes to respond in enhanced manner to subsequent agonist stimuli. For example, priming with PAF36 or TNFα37 amplified N-formyl-t-methionine-t-leucyl-t-phenylalanine (FMLP)-induced polymorphonuclear aggregation, adhesiveness, and superoxide production. In addition, TNFα was shown to prime PAF-induced superoxide production by human polymorphonuclear cells,15 while peripheral blood monocytes primed with PAF responded by secreting TNFα to both phorbol esters or concanavalin A.20 and PAF primed rat spleen macrophages to release interleukin-1 in response to LPS. PAF was also shown to elicit TNFα production from lymphocytes and macrophages,13,38 and TNFα was reported to trigger PAF synthesis from polymorphonuclear leukocytes, vascular endothelial cells, and macrophages in vitro.14

PAF primed LPS-induced hypotension, hemoconcentration, leukopenia, and thrombocytopenia. These data are compatible with a previous study39 in which rats pretreated with zymosan, an inducer of
endogenous TNFα production, exaggerated their hypertensive and leukopenic response to LPS. The role of PAF in the above-mentioned phenomenon was inferred from experimental groups where pretreatment with the PAF antagonist SRI 63-441 attenuated the response.

Thromboxane A2 (TXA2), a potent vasoconstrictor and antiplatelet aggregation factor (for review, see Reference 40), is believed to interfere with organ blood flow and to promote ischemia during endotoxic shock. Earlier in vivo studies demonstrated that endotoxin elevates plasma TXB2 levels by an unknown mechanism. Nevertheless, recent reports clearly showed that pretreatment with various PAF antagonists markedly attenuated the LPS-induced elevation of plasma TXB22,17,42 suggesting that the TXA2 response to endotoxin is partly dependent on PAF. Since in our study PAF primed for LPS-induced elevation of plasma TXB2, it is possible that priming mode is the mechanism of PAF/TXA2 dependency.

At 4 hours after injury, PAF primed for neutrophil infiltration to the lung (quantitated by measuring MPO activity) increased pulmonary water content and sequestration of fibrin-platelet aggregates. These phenomena are characteristic of LPS-43,44 and TNFα-induced45,46 lung injury and of the acute phase (12–24 hours) of the ARDS associated with clinical septic shock (for review, see Reference 32). At 24 hours after injury, the inflammatory process progressed to a chronic type of injury with leukocytic infiltrate characterized by monocyte predominance, reminiscent of the interstitial infiltrates observed in the later stages of clinical ARDS. In addition, the pulmonary response included a proportional increase in the lung dry weight, possibly indicating substantial increase in lung permeability to plasma proteins and cells, extravasation, and accumulation in the pulmonary parenchyma. It was recently suggested that TNFα induces neutrophil-dependent pulmonary edema associated with increased vascular permeability (mediated by PAF) and pulmonary capillary pressure (mediated by TXA2 and PAF46). Taken together, these data might point to the possibility that PAF could serve as a priming factor for the development of ARDS in septic states, acting at early times when plasma endotoxin levels are still extremely low.

The main characteristics of the priming process are 1) it requires the presence of PAF in the circulation. This conclusion was drawn since the injection of LPS at 120 minutes after the termination of PAF infusion, when PAF was already cleared from the circulation (half-life of <30 seconds47), did not prime for TNFα production; 2) it necessitates PAF stimulation over a minimal period of 5 to 15 minutes (since priming was demonstrated when PAF was infused for 15 minutes but not when infused for 5 minutes); and 3) it is subjected to desensitization (there was no priming after the injection of LPS at the end of PAF infusion, when PAF is still present in the circulation).

The pathophysiological pathways by which the priming effect of PAF facilitates LPS-induced lung injury and the mechanism of the priming effect of PAF on TNFα production in response to LPS are currently unknown. It is possible that PAF induces the production of TNFα in response to LPS or, alternatively, that PAF inhibits a TNFα-degrading protease(s). However, the latter possibility is unlikely, because LPS on its own did not produce TNFα. Nevertheless, several mechanisms were suggested for PAF/TNFα amplification processes in models other than endotoxin shock. For example, Braquet et al15 suggested that the amplification of superoxide production induced by PAF and primed by TNFα might involve a modification of the functional state of certain subtypes of G protein, a
modulation of intracytosolic cAMP level, and a decrease in calcium signal threshold.

Despite the many pathophysiological consequences of the priming effect, no mortality was recorded. This might be due to an insufficient exposure to endotoxin, as might be suggested by the observation that only transient hemodynamic, hematologic, and biochemical responses were elicited by the priming process, or because the counteracting mechanisms of the body are able to cope with this degree of insult. Alternatively, it is possible that other inflammatory mediators are required for the development of the full-blown septic syndrome and ARDS.

To maintain an adequate stimulus for TNFα production, PAF was infused intravenously for 60 minutes. The infusion of PAF caused hypotension, hemoconcentration, and elevation of plasma TXB2, all well-known effects of PAF when injected as a bolus (for review, see Reference 1). However, in contrast to the administration of PAF as an intravenous bolus,3,48 desensitization was developed during the second half of the infusion.
period. These data are in agreement with those reported by Ezra et al., who observed desensitization to the hypertensive response caused by the intracoronary infusion for 6 minutes of PAF (6 nmol/min) in the domestic pig. Also, Kochanek et al. reported that PAF infusion at a rate of 0.035 ml/min (67 pmol/min) into the carotid artery of anesthetized rats decreased blood pressure by 43% at 15 minutes and 37% at 60 minutes. Nevertheless, in another study conducted in anesthetized rats, PAF (0.05–0.5 μg/kg/min i.v.) infused for 20 minutes induced hemocoagulation that attained a maximum 10 minutes after the end of infusion and then decreased slowly, but was still significant 100 minutes later. The mechanisms of the development of desensitization to the PAF-induced hypotension, hemocoagulation, and elevation of plasma TXB₂ and the changes in the time course of TNFα response during prolonged PAF infusion merit further investigation. Nevertheless, sustained production, reduced clearance, or inhibition of downregulation of mRNA could serve as possible explanations of these phenomena.

In conclusion, the present study supports the concept that endotoxin exerts its biological effects through the production of multiple mediators and provides in vivo evidence that PAF primes the synthesis of TNFα, the hemodynamic consequences, and lung injury in response to endotoxin stimulation. In addition, the present study introduces a new conceptual and clinically relevant rat model of ARDS, in which the disease state results from interactions between low doses of inflammatory mediators (LPS dose required is 10³ smaller than any previous model of ARDS in the rat). This model might be useful in future studies designed to investigate septic lung injury in humans.

References


**Key Words** • adult respiratory distress syndrome • endotoxic shock • platelet-activating factor • TNFα • thromboxane • lipopolysaccharide
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