PAF receptor antagonist modulates neutrophil responses with thermal injury in vivo

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Fazal, Nadeem, Walid M. Al-Ghoul, Mashkoor A. Choudhry, and Mohammed M. Sayeed. PAF receptor antagonist modulates neutrophil responses with thermal injury in vivo. Am J Physiol Cell Physiol 281: C1310–C1317, 2001.—The role of platelet-activating factor (PAF) in Ca \(^2+\) signaling and Ca \(^2+\)-related enhancement of reactive oxygen intermediate (ROI) generation in neutrophils of burn-injured rats was ascertained by evaluating the effect of treatment of the rats with a PAF receptor antagonist. The treatment of rats with the antagonist also allowed us to evaluate the role of PAF in the priming of neutrophil ROI response with burn in vivo. A full skin thickness burn injury was produced in anesthetized rats by exposing 30% of total body surface area to 98°C water for 10 s. Sham and burn rats were killed 1 day later, and their blood was collected to obtain neutrophils. Fluorescence-activated cell sorter analysis was used to quantify ROI production by the neutrophils. Cytosolic-free Ca \(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) imaging technique was employed to measure neutrophil [Ca\(^{2+}\)]\(_i\) in individual cells and microfluorometry for the assessment of [Ca\(^{2+}\)]\(_i\) responses in suspensions of neutrophils. There was an overt enhancement of ROI generation by burn rat neutrophils. ROI release was accompanied by a marked elevation of [Ca\(^{2+}\)]\(_i\) signaling. The treatment of rats with PAF receptor antagonist before burn prevented the upregulation of both [Ca\(^{2+}\)]\(_i\) and ROI generation in neutrophils. These studies indicate that enhanced ROI production in neutrophils in the early stages after burn injury results from a PAF-mediated priming of the [Ca\(^{2+}\)]\(_i\), signaling pathways in vivo.

burn; rat; polymorphonuclear neutrophils; platelet-activating factor; reactive oxygen intermediates; calcium-protein kinase C signaling; platelet-activating factor blockade

NEUTROPHILS PLAY A CENTRAL ROLE in generating toxic oxygen radicals during inflammatory conditions. A membrane-bound multicomponent NADPH oxidase is responsible for the production of oxygen radicals. In resting neutrophils, this enzyme complex consists of unassembled plasma membrane and cytosolic components (31, 44). After neutrophil activation, the cytosolic components p40\(_{phox}\), p47\(_{phox}\), p67\(_{phox}\), and Rac-2 translocate to the membrane where they associate with flavocytochrome b558, a heterodimer comprised of gp22\(_{phox}\) and gp91\(_{phox}\), and Rap1A to form the active oxidase complex (4, 40). The respiratory burst is enhanced by a number of proinflammatory mediators, such as tumor necrosis factor (TNF)-\(\alpha\) (53), granulocyte/macrophage colony-stimulating factor (52), and lipopolysaccharide (49), which generally do not stimulate respiratory burst activity on their own. Some of the chemotactic mediators that activate NADPH oxidase and stimulate respiratory burst are activated serum complement C5a, N-formylmethionyl-leucyl-phenylalanine (fMLP), and bioactive lipids PAF and leukotriene B\(_4\) (4). The enhancement of respiratory burst activity is referred to as priming. Previous in vitro studies have shown that stimulation of resting neutrophils by a mediator results in either enhanced respiratory burst or priming of the respiratory burst mechanism for a subsequent enhanced production of \(O_2^+\) (2, 40, 57). Priming presumably occurs in vivo during injury such as burn or infection (3, 14, 47); it occurs after intravenous infusion of TNF-\(\alpha\) into human volunteers (53).

It is known that PAF levels are increased in the course of injuries such as burn, sepsis, or ischemia and reperfusion (1, 10, 11, 19, 46). PAF is released from macrophages, neutrophils, and vascular endothelial cells (9, 26, 27, 35, 36). Studies of neutrophils, which were isolated from injured animals (13, 25) or injured patients (5, 58), have shown that PAF receptor modulations were involved in the respiratory burst responses during injury. Other studies have addressed the involvement of PAF in the neutrophil priming process. In the latter studies, neutrophils from healthy volunteers were exposed to sera from trauma patients in the presence or absence of the PAF receptor antagonist (PAFra) or neutrophils from trauma patients were themselves stimulated in vitro in the presence or absence of the PAFra (5). Thus the occurrence of neutrophil priming with burn or trauma injury was primarily ascertained through in vitro experiments. A major objective of this study was to evaluate the role of PAF priming of neutrophil in vivo with burn injury. PAF that caused modulation was examined in rats treated with a PAFra (PAF-16) before subjecting them...
to a 30% total body surface area (TBSA) full-thickness burn.

Despite the implications of certain inflammatory agents contributing to neutrophil priming during the course of burn or trauma injury, little is known about the intracellular signaling mechanisms causing such priming. A role of protein kinase C (PKC) and subsequent protein phosphorylations in the O₂⁻ production of polymorphonuclear neutrophils has been well established (39, 56). It can be due either to a direct activation of the kinase by the second messenger diacylglycerol or to an increase in cytotoxic-free Ca²⁺ concentration ([Ca²⁺]<sub>i</sub>) and a related subsequent activation of the Ca²⁺-dependent PKC-β isoform (29). The Ca²⁺-dependent PKC isoform has been shown to phosphorylate the cytosolic proteins p47<sub>phox</sub> and p67<sub>phox</sub>, which are then translocated to the membrane before NADPH oxidase activation and O₂⁻ which are then translocated to the membrane before

MATERIALS AND METHODS

**Animals.** Adult male Sprague-Dawley rats weighing 250–275 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). The rats were acclimatized in the animal quarters for 3 days before use. The care of animals was in accordance with the guidelines set by Loyola University Chicago Medical Center Animal Care and Use Committee.

**Thermal injury protocol.** The animals were anesthetized with an intraperitoneal (ip) injection of pentobarbital sodium (45 mg/kg body wt). The hair on the animals’ backs was clipped off. The animals were then placed in a supine position in a plastic template that exposed 30% of the TBSA. In the sham group, the exposed backs were immersed for 10 s in a water bath at room temperature. In the burn group rats, full-thickness skin scalds were inflicted by immersing the back of the animal to 95°C (203°F) water for 10 s. Rats were quickly dried after the exposure to hot water to avoid additional injury. The animals in each group were resuscitated with 10 ml of 1% paraformaldehyde solution and the blood (10–12 ml) was collected into heparinized syringes by means of cardiac puncture. Neutrophils were isolated from the heparinized blood using the standard Ficoll-Paque (Pharmacia) cell separation technique followed by dextran sedimentation and hypotonic red blood cell lysis. Neutrophils were then washed and resuspended in HBSS (Hanks’ balanced salt solution) buffer. Neutrophil preparations routinely contained ≥95% neutrophils, as identified by the Giemsa stain, and were found to be ≥98% viable by the trypan blue exclusion technique.

**Flow cytometric assay for measurement of ROI.** Fluorescence-activated cell sorter (FACS) analysis determined the ability of neutrophils to generate an oxidative burst by indirectly measuring the increase in fluorescence generated by the oxidation by O₂⁻ of a laser-sensitive dye, dihydrorhodamine 123 (Molecular Probes, Eugene, OR) at a final concentration of 2.5 μg/ml. Dihydrorhodamine 123 was stored at a stock concentration of 5 mg/ml in N,N-dimethylformamide (Sigma, St. Louis, MO) at 70°C. After a 15-min incubation period, FMLP (1 μM) or phorbol 12-myristate 13-acetate (PMA) (100 ng/ml) was added, and the cells were incubated for an additional 15 min at 37°C in a shaking water bath. After the last incubation, the samples were centrifuged at 400 g for 5 min, and the supernatant was discarded. The pellet was resuspended in 2 ml of erythrocyte lysing solution and was allowed to stand at room temperature for 10 min. The cells were then washed in a solution containing 2.5% sodium azide and 10% fetal bovine serum (GIBCO, Grand Island, NY) in PBS and resuspended in 0.7 ml of 1% paraformaldehyde solution. After fixation, samples were acquired on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). The machine is equipped with an argon ion air-cooled laser that emits a peak line of fluorescence of 488 nm at 15 mW. At least 10,000 events were acquired by using LYSIS II software (Becton Dickinson). Both forward-angle and right-angle light scatter signals were acquired by using LYSYS II software (Becton Dickinson). The forward-scatter threshold signal was adjusted to exclude debris and unlysed erythrocytes. All parameters were optimized on unstimulated purified neutrophils that were loaded with dihydrorhodamine 123. The flow cytometry/dihydrorhodamine technique proved useful for studying rat neutrophils because it could be used with relatively small numbers of cells (1 × 10⁶). Furthermore, a number of reactive oxygen species can oxidize dihydrorhodamine (such as hydrogen peroxide, peroxynitrite, and peroxynitrite) (34). It is known that rat neutrophils preferentially generate intracellular oxidants, even in response to soluble stimuli. Because H₂O₂ is considerably more membrane permeable than O₂⁻, this suggests that H₂O₂ may be more important for oxidant-dependent neutrophil cytotoxicity in rat.

**Calcium measurements in cell suspensions.** The [Ca²⁺]<sub>i</sub> in suspension of neutrophils was determined by microfluorometry. Neutrophils (3 × 10⁶ cells) were loaded with 2 μM fura 2-AM (Molecular Probes). After being washed, the cells were resuspended in HBSS (GIBCO RBL), and the fluorescence signals of a 0.5-ml stirred neutrophil suspension were monitored in an F-2000 Hitachi spectrofluorometer using 340- and 380-nm excitation wavelengths and a 510-nm emission wavelength. The fluorescence ratio (R = F₃₄₀/F₃₈₀) was calculated and converted to [Ca²⁺]<sub>i</sub> using the equation described by Grynkiewicz et al. (21): [Ca²⁺]<sub>i</sub> = K₅0(R - Rₐmin)/(Rₐmax - R), where Rₐmax = F₃₄₀/F₃₈₀ (with Ca²⁺), Rₐmin = F₃₄₀/F₃₈₀
Increases in MCF were evident in FITC-labeled sham unlabeled cell (M1) was taken as the gating control. 

Figure 1 shows representative FACS analyses after dihydrorhodamine 123 labeling of purified neutrophils. MCF of an shows representative FACS analyses after dihydrorhodamine 123 labeling of FMLP- or PMA-stimulated neutrophils. Figure 1

Production was measured in units of mean channel fluorescence (MCF) of dihydrorhodamine 123 labeling before and after stimulation with FMLP (1 μM). In neutrophils from the burn group (130 ± 10 nM, means ± SE) was significantly higher (P < 0.01) than in the sham group (100 ± 7 nM). The FMLP-induced peak [Ca2+]i in neutrophils from burn rats (480 ± 11 nM) was also significantly higher (P < 0.05) than the [Ca2+]i, response to FMLP in the sham rats (320 ± 12 nM). In the sham rat neutrophils, there was no effect of PAFra treatment on the FMLP-mediated peak [Ca2+]i (P > 0.05), but there seemed to be an increase in the basal [Ca2+]i after the treatment (P < 0.05). The FMLP-mediated peak [Ca2+]i, in the PAFra-treated burn rats was significantly less (P < 0.05) than in the untreated burn group. There was no demonstrable effect of PAFra treatment (P > 0.05) on the basal [Ca2+]i in the burn rats. The difference between the basal and the FMLP-induced peak [Ca2+]i levels (Δ[Ca2+]i) in the burn group (355 ± 11) was significantly higher (P < 0.05) compared with Δ[Ca2+]i values in the sham groups with (230 ± 9) and without (235 ± 4) PAFra treatment (Fig. 3). The pretreatment of burn rats with PAFra caused a significant (P < 0.05) attenuation of Δ[Ca2+]i response (155 ± 8).

The heightened [Ca2+]i response with FMLP in neutrophils suspensions from the burn group relative to the sham group was confirmed by [Ca2+]i measurements via Ca2+ imaging of individual neutrophils (Fig. 4). Figure 4 shows both the pseudocolor-digitized Ca2+ images and digitized fluorescent ratios (F340/F380) obtained in neutrophils from a representative sham and a representative burn rat. The frequency of cells showing pseudoblue and pseudogreen color, representing quiescent resting cells, seemed to be comparable in the sham-, burn-, and PAFra-treated burn animals. Upon stimulation with FMLP, a significant number of sham rat cells transformed into cells with pseudocolors in the yellow and red range, corresponding to an image ratio of ~1.3. The FMLP stimulation of burn rat neutrophils clearly caused their transformation into the pseudocolors red and white range, corresponding to the image ratio of >2. Imaging of individual neutrophils confirmed that FMLP-induced [Ca2+]i elevations in the burn group were markedly higher than cells in the sham group. Figure 4 also shows the elevation in the digitized fluorescent ratios, representing [Ca2+]i, as a function of time after FMLP stimulation of neutrophils from sham and burn rats with and without PAFra treatment. Although the Ca2+ image analyses did not show a measurable effect of treatment with PAFra on the basal [Ca2+]i, or FMLP-mediated [Ca2+]i, elevation in the sham animals, these analyses in burn rat neutrophils showed a pronounced effect of PAFra treatment on FMLP-mediated [Ca2+]i responses but not on basal [Ca2+]i. The image analyses reinforce that PAFra

Statistical analysis. The data were analyzed using the Sigma Statistical program (SPSS, version 2.0; SigmaStat). P < 0.05 between two groups was considered statistically significant.

RESULTS

ROI generated subsequent to activation of the NADPH oxidase system were measured in neutrophils before and after stimulation with FMLP (1 μM) or PMA (100 ng/ml) via flow cytometric analyses. ROI production was measured in units of mean channel fluorescence (MCF) of dihydrorhodamine 123 labeling of FMLP- or PMA-stimulated neutrophils. Figure 1 shows representative FACS analyses after dihydrorhodamine 123 labeling of puriﬁed neutrophils. MCF of an unlabeled cell (M1) was taken as the gating control. Increases in MCF were evident in FITC-labeled sham animal neutrophils stimulated with FMLP (Fig. 1A, left) or PMA (Fig. 1A, right) over burn animal neutrophils (Fig. 1B, left, or Fig. 1B, right). Nonstimulated FITC-labeled cells from sham or burn animals did not show any detectable dihydrorhodamine 123 uptake, indicating little ROI production (data not shown). PAFra treatment of burned animals signiﬁcantly attenuated the dihydrorhodamine 123 labeling of neutrophils stimulated with FMLP (Fig. 1C, left) or PMA (Fig. 1C, right), compared with the labeling in the untreated burn neutrophils.

Figure 2 shows pooled MCF values from at least 10 animals in the sham or burn group. The peak ROI production by burn rat neutrophils stimulated with PMA (75 ± 4 MCF, means ± SE) or FMLP (70 ± 3 MCF) was signiﬁcantly higher (P < 0.05) than in the sham (PMA, 45 ± 3; FMLP, 35 ± 2) or the PAFra burn group (PMA, 50 ± 4; FMLP, 45 ± 4). There was no signiﬁcant difference between the peak ROI production in the sham- or PAFra-treated burn rats after PMA or FMLP stimulation (Fig. 2, A and B). Thus PAFra pretreatment effectively prevented the burn-caused enhancement of neutrophil ROI production.

The [Ca2+]i measurements in suspensions of neutrophils, from sham and burn rats, are shown in Fig. 3. The burn injury caused elevations in both basal [Ca2+]i, and FMLP-mediated [Ca2+]i, responses. The basal [Ca2+]i in neutrophils from the burn group (130 ± 10 nM, means ± SE) was significantly higher (P < 0.01) than in the sham group (100 ± 7 nM). The FMLP-induced peak [Ca2+]i, in neutrophils from burn rats (480 ± 11 nM) was also significantly higher (P < 0.05) than the [Ca2+]i, response to FMLP in the sham rats (320 ± 12 nM). In the sham rat neutrophils, there was no effect of PAFra treatment on the FMLP-mediated peak [Ca2+]i (P > 0.05), but there seemed to be an increase in the basal [Ca2+]i after the treatment (P < 0.05). The FMLP-mediated peak [Ca2+]i, in the PAFra-treated burn rats was significantly less (P < 0.05) than in the untreated burn group. There was no demonstrable effect of PAFra treatment (P > 0.05) on the basal [Ca2+]i in the burn rats. The difference between the basal and the FMLP-induced peak [Ca2+]i levels (Δ[Ca2+]i) in the burn group (355 ± 11) was significantly higher (P < 0.05) compared with Δ[Ca2+]i values in the sham groups with (230 ± 9) and without (235 ± 4) PAFra treatment (Fig. 3). The pretreatment of burn rats with PAFra caused a significant (P < 0.05) attenuation of Δ[Ca2+]i response (155 ± 8).

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treatment of burn rats led to a marked attenuation of [Ca^{2+}]_i responses to FMLP.

**DISCUSSION**

In the present investigation, we obtained evidence that PAF contributes to hyperactivation of circulating neutrophils in vivo in an early stage of inflammation following burn, namely, day 1 postburn. The hyperactivation of neutrophils is evident from enhanced ROI production in conjunction with upregulated Ca^{2+} signaling, which is essential for the respiratory burst through NADPH oxidase activation (4, 22, 42, 54). PAF is known for its ability to enhance adherence, chemotaxis, and respiratory burst of neutrophils (9, 28, 37, 38, 41, 48, 50). Clinical and laboratory studies have demonstrated that PAF metabolites are released in circulation during inflammatory conditions such as acute respiratory distress syndrome, burn, trauma, sepsis, and intestinal ischemia-reperfusion (1, 10, 11, 19, 20).
The stimulation of neutrophils with PMA would be expected to result in a potentially maximal level of ROI production, due presumably to a direct maximal stimulation of PKC and downstream signaling to NADPH oxidase (15, 23, 32, 51, 56). The higher level of ROI production with PMA than with FMLP in the sham rat neutrophils (Fig. 2) supports the concept that FMLP contributes only partly to the activation of PKC. The neutrophils in sham rats were likely not exposed in vivo to neutrophil-activating chemotactic mediators such as would be found in the burn-injured rats. Consequently, there is little probability of their prior in vivo “priming” and/or activation by such mediators. Thus the partial activation of sham rat neutrophils’ oxidant response by FMLP, relative to that by PMA, might represent neutrophils’ innate responses without their prior priming or activation by the mediators. In the burn rat neutrophils, there was not only an absence of a difference in the effects of PMA and FMLP on ROI production but also a significantly higher level of ROI production with either PMA or FMLP, compared with that in the sham rat neutrophils. The increase in ROI production with PMA in burn injury over the PMA-mediated ROI production in the sham group suggests that a burn inflammatory condition caused an upregulation of PKC signaling, leading to high ROI production. The upregulation of PMA-mediated ROI in burn injury could possibly result from an in vivo overactivation of signaling events proximal to PKC. The fact that blockade of endogenous PAF in the burn animals effectively attenuated PMA caused ROI production to the level found in the sham animal neutrophils supports that PAF could modulate PKC signaling. A possible action of PAF released after burn could be priming of neutrophils in vivo such that a secondary action of a neutrophil-activating agent, e.g., exogenous PMA or FMLP, would produce a potentiated production of ROI compared with that occurring in neutrophils without priming. The absence of the difference in the effects of PMA and FMLP on ROI production by a burn rat neutrophil could also be related to endogenous neutrophil priming via actions of burn-related PAF. Priming per se may upregulate signaling such that a subsequent activation of neutrophils via FMLP leads to ROI production equal to that obtained with PMA. Previous in vitro studies have shown that when neutrophils were primed with PAF and subsequently stimulated with FMLP, the neutrophil O2 production occurred at a maximal rate (23, 28).

In neutrophils, a key signaling step either parallel or upstream to PKC activation is [Ca2+]i mobilization (23, 24). Because the Ca2+-dependent PKC isoform, PKC-β, seems to be abundant in neutrophils (29), it is reasonable to assume that [Ca2+]i elevation could play a role in PKC activation. However, there is also evidence that PKC activation could occur via Ca2+-independent upstream signaling events (17, 24). The greater magnitude of the [Ca2+]i response to FMLP in the burn rat neutrophils than that in the sham rat neutrophils (Fig. 3) may be responsible for the higher potentiated PKC activation and consequent ROI production. This consideration allows us to conclude that PAF blockade in burn-injured rats may prevent the upregulation of the Ca2+-linked PKC signaling pathway to prevent the potentiated neutrophil ROI response. A number of previous studies have implicated a role of PAF in the

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**Fig. 2.** A and B: ROI production response to FMLP (A) and PMA (B) in neutrophils harvested from sham, sham+PAFra-treated, burn, and burn+PAFra-treated rats. Bar graphs represent means ± SE (n = 10) MCF values obtained from flow cytometry analyses using dihydrorhodamine 123 labeling; n, number of animals; ⁎P < 0.05 sham vs. burn or PAFra-treated animals.

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**Fig. 3.** Cytosolic-free Ca2+ concentration ([Ca2+]i) response to FMLP in neutrophils harvested from sham, sham+PAFra-treated, burn, and burn+PAFra-treated rats. Bar graphs represent means ± SE (n = 10) values of [Ca2+]i, in nanomolar units; n, number of animals; ⁎P < 0.05, sham vs. burn or PAFra-treated animals. ⁎⁎P < 0.01, sham vs. sham-or burn-PAFra-treated animals. #P > 0.05, sham vs. sham+PAFra. Open bars, basal; hatched bars, FMLP; and solid bars, difference between basal and FMLP-stimulated values.
priming and/or activation of circulating neutrophils during burn/trauma injury conditions (1, 10–13, 35, 36, 43, 50). However, these studies assessed the PAF role primarily via measurements in isolated neutrophils from healthy human volunteers and from injured patients or animals with such injuries, incubated with PAF in the absence and/or presence of the PAFra. In other studies, the investigators demonstrated neutrophil priming with burn via measurements in healthy human volunteer blood neutrophils incubated with plasma from burn patients along with PAFra in vitro (5). The present study reinforces the role of PAF in blood neutrophil priming via endogenous PAF blockade in vivo in burn-injured animals.

Our present finding of an increase in neutrophil basal [Ca^{2+}], with burn injury is in agreement with previous studies (39). This increase was interpreted to indicate an upregulation of Ca^{2+} signaling with burn injury in vivo. In this study, although PAFra effectively abrogated FMLP-mediated [Ca^{2+}] response, it did not have an effect on the burn-induced increase in basal [Ca^{2+}]. This implies that while endogenous PAFra could modulate Ca^{2+} signaling triggered by exogenous FMLP in the presumably burn-preprimed neutrophils,
it could not prevent an endogenous activation of neutrophil Ca\(^{2+}\) signaling reflective in the measured level of basal \([\text{Ca}^{2+}]_i\). It is plausible that such endogenous Ca\(^{2+}\) signaling activation in burn rat neutrophils may not be related to endogenous PAF action. The lack of effect of PAFra on the basal \([\text{Ca}^{2+}]_i\) increase in burn rat neutrophils may also be related to the observed decrease in the \(\Delta\text{Ca}^{2+}\) response (FMLP-mediated \([\text{Ca}^{2+}]_i\) – basal \([\text{Ca}^{2+}]_i\)) below the level of \(\Delta\text{[Ca}^{2+}]_i\) response in the sham rat neutrophils. This is accounted by the observed decrease in \(\Delta\text{Ca}^{2+}\) (sham vs. PAFra-treated burn) in the absence of a difference between the FMLP-mediated peak \([\text{Ca}^{2+}]_i\) responses in the sham and PAFra-treated burn rat neutrophils.

Several studies have suggested that early neutrophil hyperactivation following burn, trauma, and ischemia-reperfusion episodes contributes to the development of multiple organ failure (2, 3, 14, 32). PAF release is associated with hemodynamic instability and organ or cellular dysfunction in a wide range of human (1, 10, 19, 20, 33, 58) and animal (11, 18) conditions of systemic inflammation. Previous studies have also indicated that PAFra, administered early in gram-negative bacteremia or endotoxemia in rodents, reduces pulmonary hypertension and capillary leak (13). These findings implicate PAF as a significant early mediator of pulmonary hypertension and capillary leak (13). These findings may also be related to the observed effect of PAFra on the basal \([\text{Ca}^{2+}]_i\) increase in burn rat neutrophils. This is consistent with the observed decrease in \(\Delta\text{Ca}^{2+}\) (sham vs. PAFra-treated burn) in the absence of a difference between the FMLP-mediated peak \([\text{Ca}^{2+}]_i\) responses in the sham and PAFra-treated burn rat neutrophils.

PAF-mediated priming of neutrophils following postischemic gut injury in patients has been shown to be related to decreased circulating levels of PAF-acetylhydrolase (PAF-AH), which hydrolyzes PAF to lyso-PAF (35). Furthermore, reduced levels of PAF-AH have been associated with the development of multiple organ failure (MOF) (35). Other findings suggest that endogenous PAF and interleukin (IL)-8 sequentially prime neutrophils in patients with trauma at the risk of MOF (5). Plasma of such patients, when treated with PAF antagonist, inhibited priming of neutrophils and blocked their capacity to release superoxide (5). Studies have also associated PAF production in an acute phase of injury with an increase in neutrophil cytosolic Ca\(^{2+}\) (7). In these studies, the investigators found IL-6 and PAF via activation of phospholipase A\(_2\) primed neutrophils in the gut (7). However, IL-6 alone could not directly prime neutrophils for superoxide release; it synergized with PAF in vitro to prime neutrophils (8). It has been hypothesized that ischemia of gut (6) or gut ischemia-reperfusion injury (8) serves as a priming bed for circulating neutrophils which then initiates MOF.

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