MICROBIOLOGY AND IMMUNOLOGY

Role of Platelet Activating Factor in Regulation of Phagocytic Function of Macrophages in Different Organs

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Platelet activating factor diminished phagocytic activity of peritoneal macrophages (desensitizing effect) and stimulates it in splenic macrophages (priming effect). Homologous endogenous transfer factor primed resident macrophages to subsequent exposure to platelet activating factor, while heterologous factor desensitized them. Thus, desensitization effect caused by transfer factor observed after lipopolysaccharide injection *in vivo* was due to mutual effects of peritoneal and splenic macrophage populations. Therefore, an organism surviving after lipopolysaccharide injection possesses mechanisms limiting uncontrolled stimulation of macrophage function. These mechanisms are realized by down-regulation of macrophage populations by heterologous transfer factors.

Key Words: *platelet-activating factor; macrophages; phagocytosis*

Platelet activating factor (PAF) is a phospholipid with a wide spectrum of biological activity. PAF is a key component of inflammation, hypersensitivity reactions, and shock [5,8,9,13]. Recently it was reported that it modulates cellular immune response [4,5]. PAF is produced by natural killer cells, lymphocytes, platelets, endothelial cells, neutrophils, and macrophages [3,12]. Many cells not only produce PAF in response to various stimuli, but due to PAF-specific receptors exert multiple biochemical and functional effects. Macrophages belong to such cells [3,4,11,13,14]. Macrophagal population is heterogeneous; PAF production and expression of PAF-specific receptors vary within a wide range. Therefore, functional response of macrophages to PAF is not uniform, which does not rule out the probability of its regulatory effect at the level of macroorganism.

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We studied the role of PAF in the regulation of phagocytic function of macrophages in different populations exposed to endotoxic shock.

MATERIALS AND METHODS

Male C57Bl/6 mice weighing 18-20 g were used. Macrophages were obtained from animals injected with 1) *Pseudomonas aeruginosa* lipopolysaccharide (LPS) in doses of 0.3 mg (LD_{50}) or 0.15 mg (suboptimal dose) in 0.1 ml normal saline intravenously into the retroorbital sinus or 2) PAF or lysoPAF in a dose of 10^{-5} M in 1 ml normal saline intraperitoneally 1 day before testing. Intact animals were used as controls.

Macrophage monolayer was prepared by incubation of peritoneal exudate cells and splenocytes [2] with heparin for 1 h at 37°C. PAF was added to the monolayer in a dose of 10^{-6} M/dish and incubated for 30 min, after which the monolayer was thoroughly washed.

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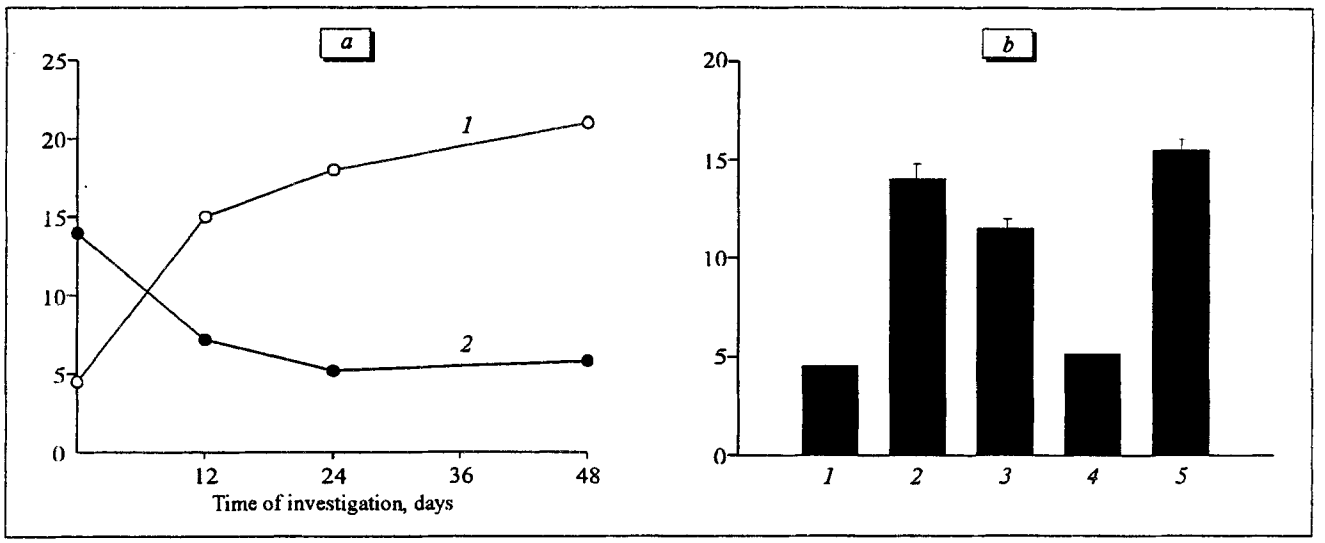

Fig. 1. Effect of platelet activating factor (PAF) on phagocytic function of peritoneal and splenic macrophages activated *in vivo* with lipopolysaccharide (LPS) (*a*) and PAF (*b*). *a*) *1*: LPS, 0.3 mg *in vivo*; *2*: LPS *in vivo* + PAF, 10^{-6} M *in vitro*. Crossing of curve *1* with the ordinate corresponds to intact control, crossing of curve *2* with the ordinate to phagocytic activity of macrophages under the effect of PAF, 10^{-6} M *in vitro*. *b*) *1*: intact macrophages; *2*: PAF, 10^{-5} M *in vivo*; *3*: PAF, 10^{-6} *in vivo* + PAF, 10^{-6} M *in vitro*; *4*: lysoPAF, 10^{-5} M *in vivo*; *5*: lysoPAF *in vivo* + PAF, 10^{-6} M *in vitro*. Here and in Figures *2* and *3*: ordinate: phagocytosis of ^{14}C-labeled typhoid fever vaccine, cpm/mg protein $\times 10^3$.

For assessing the phagocytic function, 1 ml medium 199 and 0.1 ml 2-billion suspension of ^{14}C-labeled typhoid fever vaccine were added to the resultant monolayer and incubated at 37°C for 1 h. The monolayer was washed and resuspended in 0.5 ml normal saline. Radioactivity and protein concentration were measured in the resultant cell suspension. Results were expressed in cpm/mg protein.

In one series of experiments we used a supernatant, later referred to as transfer factor. The supernatant was prepared by culturing macrophages of mice pretreated with suboptimal LPS dose. The supernatant was centrifuged at 600*g* for 15 min and 1 ml of it was added to a monolayer of intact peritoneal exudate and splenic macrophages (PM and SM, respectively) for 30 min. After incubation the monolayer was washed and treated with PAF. Subsequent treatment was performed as described above.

The significance of differences was evaluated using Student's test.

RESULTS

In the first series we studied the effect of PAF (10^{-6} M) on phagocytic function of PM of mice survived after LD_{50} of LPS. Macrophages were obtained 12, 24, and 48 days after LPS injection. Figure 1, *a*, shows that macrophagal phagocytic activity increases with time after injection of LPS. Treatment with PAF *in vitro* decreased phagocytosis at all terms studied. Since PAF is one of the major mediators of endotoxin shock, it can be expected that stimulation of phagocytosis after a high dose of LPS in mice was caused by the action of PAF on macrophages. A decrease in phagocytic activity of macrophages observed later resulted from repeated treatment with a high dose of PAF. It is noteworthy that inhibition of phagocytosis did not result from cytotoxic effect of PAF, because baseline activity under the effect of PAF *in vitro* was higher than in intact cells (Fig. 1, *a*). In addition, Trypan Blue test failed to detect the cytotoxic effect of PAF. The inhibition of phagocytosis may result from specific desensitization of macrophages. To confirm this hypothesis, PAF or lysoPAF were used as the initial and PAF as the second stimuli. Treatment of macrophages with PAF *in vivo* led to stimulation of phagocytosis in comparison with intact controls (Fig. 1, *b*). Additional exposure of macrophages to PAF *in vitro* caused a drastic descrease in phagocytic activity. In contrast to this, re-exposure to PAF *in vitro* after initial exposure to lysoPAF *in vivo* stimulated phagocytic activity. Thus, only treatment with PAF desensitized macrophages to its repeated exposure.

Since macrophage population is heterogeneous, PM and SP responses to PAF are different. In the second series of experiments we investigated the effect of PAF (10^{-7} M) on phagocytic function of PM and SM obtained 6 days after injection of suboptimal LPS. Figure 2 shows that for PM, neither PAF in a dose of 10^{-7} M, nor LPS in a dose of 0.15 mg causes any changes in phagocytosis. PAF decreased phagocytic activity of macrophages activated

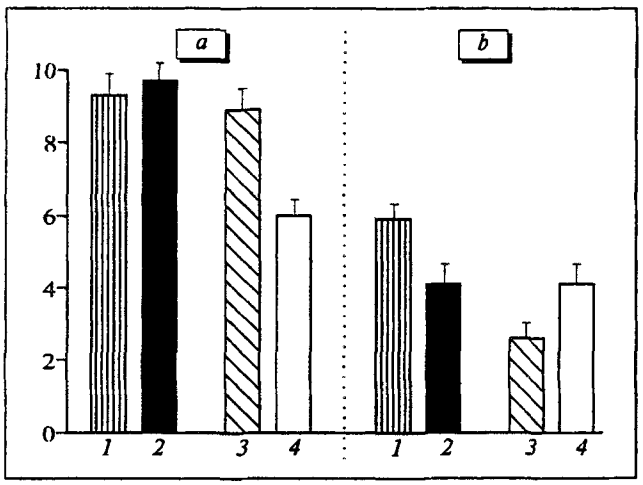

Fig. 2. Effect of platelet activating factor (PAF) on phagocytic function of peritoneal (*a*) and splenic (*b*) macrophages activated *in vivo* with lipopolysaccharide (LPS) in suboptimal dose. *1*) intact macrophages; 2) PAF, 10^{-7} M *in vitro*; 3) LPS, 0.15 mg *in vivo*; *4*) LPS *in vivo* + PAF *in vitro*.

by LPS. By contrast to PM, priming effect was observed in SM.

It is impossible to assess mutual effects of the studied macrophage populations *in vivo*, and therefore we carried out experiments with transfer factor *in vitro*. A monolayer of resident intact SM and PM was incubated with transfer factor. Each population of intact macrophages was treated with homologous and heterologous transfer factor. Figure 3, *a*, illustrates the phagocytic response of PM to PAF after pretreatment with transfer factor obtained from PM and SM. A decrease in phagocytic response to PAF

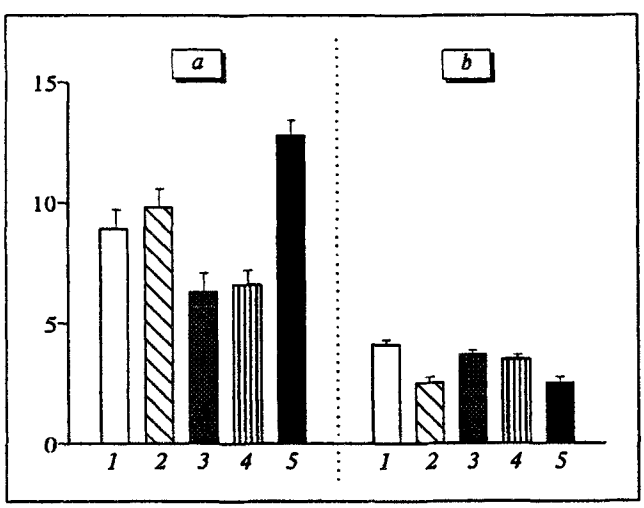

Fig. 3. Effect of platelet activating factor (PAF) on phagocytic function of peritoneal (*a*) and splenic (*b*) macrophages pretreated *in vitro* with homologous and heterologous transfer factor. *1*) PAF, 10^{-7} M *in vitro*; 2) transfer factor obtained from splenic macrophages; 3) the same + PAF; *4*) transfer factor from peritoneal macrophages; *5*) the same + PAF.

was observed after initial treatment of macrophages with the factor prepared from SM. Cells pretreated with transfer factor from PM stimulated phagocytosis in response to PAF. Figure 3, *b*, presents the results of a similar experiment on intact SM. Cell response to PAF was similar to that shown in Fig. 3, *a*. Hence, PAF exerted opposite effects on the phagocytic functions of PM and SM, acting as a modifier of phagocytic response at the level of the host.

By activating macrophages, LPS stimulates production of PAF, expression of PAF-specific receptors, and functional activity of macrophages [4,10,11,14]. We observed activation of phagocytosis in PM after injection of a high dose of LPS. How-ever, it was followed by inhibition of phagocytosis after subsequent exposure to exogenous PAF. Pre-sumably, specific desensitization detected in PM is an instrument limiting unregulated stimulation of phagocytosis in them. In SM population, injection of LPS to mice decreased phagocytosis. The activity of intracellular PAF-acetyl hydrolase in macrophages increases during maturation [15], resulting in a lower capacity of a cell to accumulate PAF. The ability to accumulate PAF is probably decreased in SM as in more mature cells, which can be a causes of phagocytosis inhibition after their exposure to LPS. Under such conditions, exogenous PAF, not completely destroyed by acetyl hydrolase, stimulated phagocytosis in SM (Fig. 2). The ability to accumulate PAF is apparently much higher in activated PM than in SM. Therefore, the observed desensitization effect is probably due to triggering of the down-regulation mechanism of PAF receptor expression due to its excessive concentration. Another possible cause is decreased affinity of PAF receptor.

Experiments with transfer factor showed that macrophages of different origin release factors differently affecting phagocytic activity of macrophages in these organs in response to LPS. Treatment with homologous endogenous transfer factor primed resident macrophages to subsequent exposure to PAF, stimulating the phagocytic response. Treatment with heterologous transfer factor desensitized resident macrophages to subsequent exposure to PAF. The use of transfer factor *in vitro* showed that desensitization after LPS addition is realized at the expense of mutual effects of PM and SM populations.

From our results it can be concluded that a macroorganism surviving after exposure to LPS possesses mechanisms limiting the uncontrollable stimulation of macrophagal function, which prevents their exhaustion. These mechanisms are realized by down-regulation of heterogeneous populations by transfer factors. In our experiments it was clearly seen in PM and SM populations.

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