Role of Eicosanoids in Regulation of Macrophage Phagocytic Functions by Platelet-Activating Factor during Endotoxic Shock

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We studied the role of eicosanoids in the regulation of macrophage phagocytic functions by products secreted in heterogeneous populations of macrophages and platelet-activating factor during endotoxic shock. Phagocytic activity depended on the metabolism of arachidonic acid in target macrophages and the ratio between its cyclooxygenase and lipoxygenase metabolites produced by heterogeneous populations of macrophages and affecting target cells. The regulatory effect of platelet-activating factor on phagocytosis was related to its interaction with products of the arachidonic acid cascade. Depending on the quantitative ratio of eicosanoids, platelet-activating factor produced various effects on phagocytic functions of heterogeneous macrophage populations.

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

Our previous studies showed that under conditions of endotoxic shock, tissue macrophages secrete mediators producing various effects on intact macrophages [1]. *In vitro* experiments with transfer factor demonstrated the interaction between peritoneal and spleen macrophages and their contribution in the modulation of phagocytic activity of target macrophages and showed the role of platelet-activating factor (PAF) in these processes. In the macroorganism survived after exposure to lipopolysaccharide, there are some mechanisms preventing excessive activation of phagocytosis and macrophage exhaustion. These mechanisms involve PAF and macrophage-derived transfer factors.

Apart from PAF, eicosanoids, the products of cyclo- and lipoxygenase (COP and LOP, respectively) metabolic pathways of arachidonic acid, play a role in the pathogenesis of endotoxic shock [5,7]. Their role in these processes is of considerable interest.

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Here we studied the role of arachidonic cascade products in the regulation of macrophage phagocytic functions by transfer factors and PAF at the end of the acute phase of endotoxic shock.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice weighing 18-20 g. To study the role of products secreted by macrophages in the regulation of their phagocytic functions during endotoxic shock, we used supernatants obtained by culturing of peritoneal and spleen macrophages from animals intravenously injected with *Pseudomonas aeruginosa* lipopolysaccharide in a sublethal dose of 0.15 mg 7 days before the experiment. The supernatant was centrifuged at 600*g* for 15 min and added (1 ml) to the macrophage monolayer.

The macrophage monolayer was obtained by culturing cells of peritoneal exudate and spleen in medium 199 containing heparin at 37°C for 1 h [2]. The intact peritoneal macrophage monolayer was treated with the cyclooxygenase blocker indomethacin (10^{-8} M) or

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lipoxygenase blocker nordihydroguaiaretic acid (NDGA, 10^{-5} M) for 30 min. The monolayer was thoroughly washed, and the supernatant and 10^{-6} M PAF were successively added for 30 min. Phagocytic functions of peritoneal macrophages were determined using ^{14}C-labeled typhoid vaccine [1].

The results were analyzed by Student's *t* test.

RESULTS

Treatment of intact peritoneal macrophages with the homologous supernatant sharply inhibited phagocytosis (Fig. 1). Cyclo- and lipoxygenase blockers abolished this effect and enhanced phagocytosis to a level typical of intact cells (Fig. 1). Treatment of intact peritoneal macrophages with the supernatant of spleen macrophages slightly stimulated phagocytosis (Fig. 1). Indomethacin decreased phagocytic activity of peritoneal macrophages, while pretreatment with NDGA suppressed phagocytosis to a level typical of intact cells. Thus, the effects of heterologous factors secreted by bacterial lipopolysaccharide-treated macrophages on phagocytosis in target cell are mediated by arachidonic acid metabolites. It is known that toxins induce the synthesis and release of eicosanoids (prostaglandins and leukotrienes) in macrophages [3]. The COP/LOP ratio in various populations of macrophages, in particular, peritoneal and spleen macrophages, is different [8,9]. Spleen macrophages synthesize greater amounts of prostaglandin E_2 (by several times) and much less leukotriene C and hydroxyeicosatetraenoic acid than peritoneal macrophages. The data suggest that the effects of test supernatants depended on the COP/LOP ratio. The contents of COP and LOP were high in the supernatant obtained from *in vivo* activated peritoneal macrophages. In the supernatant of spleen macrophages, the content of COP (e.g., prostaglandin E_2) increased. Therefore, treatment of intact peritoneal macrophages with the homologous supernatant did not change the COP/LOP ratio, while the supernatant obtained from *in vivo* activated spleen macrophages shifted this parameter towards COP. This assumption was confirmed by our experiments with cyclo- and lipoxygenase blockers. Indomethacin and NDGA shifted the COP/LPO ratio in intact peritoneal macrophages towards LOP and COP, respectively. Homologous supernatant did not restore the equilibrium shifted by inhibitors; treatment with heterologous supernatant in the absence of inhibitors also was ineffective. After indomethacin-induced inhibition of cyclooxygenase in target macrophages, the addition of exogenous COP from the supernatant of spleen macrophages restored the COP/LOP ratio to the control level. In this case, phagocytosis decreased (similarly to the effects of peritoneal macrophage supernatant without inhibitors, Fig. 1).

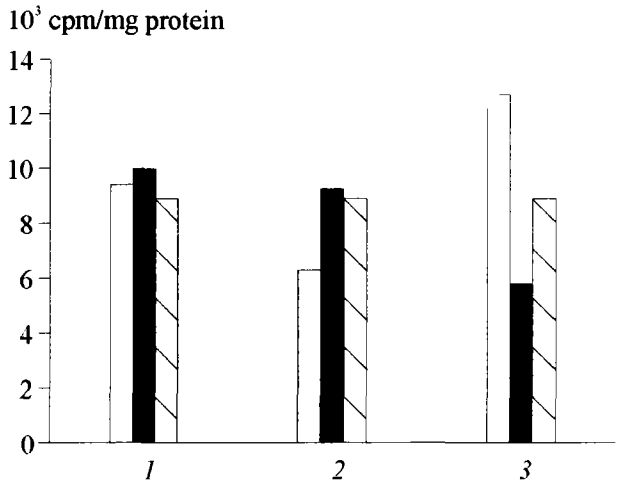

Fig. 1. Effect of transfer factor on phagocytic functions of peritoneal macrophages after inhibition of cyclo- and lipoxygenase: control (*1*) and treatment with homologous (*2*) and heterologous supernatants (*3*). Here and in Fig. 2: ordinate: phagocytic activity. Intact cells (light bars) and cells treated with indomethacin (dark bars) and nordihydroguaiaretic acid (shaded bars).

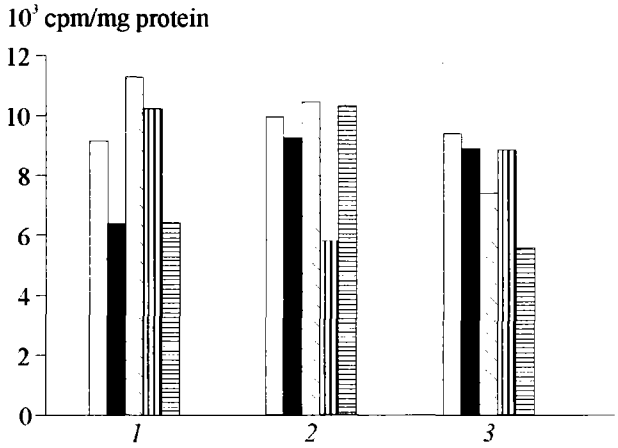

Fig. 2. Effect of platelet-activating factor (PAF) on phagocytic functions of peritoneal macrophages *in vitro* treated with transfer factor (TF) after inhibition of arachidonic acid cascade: intact cells (*1*) and treatment with indomethacin (*2*) and nordihydroguaiaretic acid (*3*). Treatment with PAF (light bars), homologous TF (dark bars), homologous TF and PAF (slant shading), heterologous TF (vertical shading), and heterologous TF and PAF (horizontal shading).

Hence, the effects of test supernatants on phagocytic activity of peritoneal macrophages depended on the COP/LOP ratio. In peritoneal macrophages, the toxin intensified production of both COP and LOP, while in spleen macrophages it stimulated only COP formation. The ultimate effects of these arachidonic acid metabolites depend on the state of target macrophages in the heterogeneous population. Enhanced production of COP stimulated phagocytic activity, while restoration of the COP/LOP ratio decreases phagocytosis.

The metabolism of arachidonic acid is closely related to PAF metabolism [10]. It was shown that PAF

stimulates the release of leukotriene B4 [4,6], while PAF production is competitively inhibited by prostaglandin E_2 [5,11]. Therefore, mutual effects of these producers on phagocytic activity of macrophages play a role under conditions of endotoxic shock. PAF-induced phagocytic response of peritoneal macrophages pretreated with heterologous supernatants was opposite to the effects of these supernatants (Fig. 2). Pretreatment with indomethacin did not change phagocytic activity of cells in response to PAF against the background of addition of homologous supernatant, but restored this parameter in the case of heterologous supernatant (Fig. 2). Under conditions of lipoxygenase blockade, PAF-induced phagocytic response of cells pretreated with homologous supernatant markedly decreased, while heterologous supernatant had no effects on phagocytic activity (Fig. 2). Therefore, the increase in phagocytic activity of cells pretreated with homologous supernatant in response to PAF was similar to changes caused by PAF against the background of pretreatment with the heterologous supernatant and inhibition of cyclooxygenase (*i.e.*, at constant COP/LO ratio). PAF-induced inhibition of phagocytosis after pretreatment with the supernatant of spleen macrophages is similar to the effects of PAF on cells pretreated with this supernatant after inhibition of lipoxygenase (*i. e.* enriched with COP, Fig. 2). Thus, the effects of PAF depend on the COP/LOP ratio in cells regulated by products of cell-to-cell interaction. Under conditions of endotoxic shock, PAF regulates phagocytic activity of macrophages by modulating the content of cyclo- and lipoxygenase arachidonic acid metabolites.

Our findings indicate that macrophages of the peritoneal exudate and spleen demonstrate various responses to lipopolysaccharide under conditions of endotoxic shock. The interaction between these macrophages is mediated by products of their secretory activity, which regulate the intensity of phagocytosis in target macrophages. Marked differences in the synthesis of eicosanoids by studied macrophages contribute to their phagocytic functions and cause functional changes in cells. The regulatory effect of PAF is related to its interaction with products of the arachidonic cascade. Depending on the COP/LOP ratio, PAF regulates the phagocytic response of macrophages in the whole body during the acute and delayed phases of endotoxic shock.

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