Role of Extracellular and Intracellular Nitric Oxide in the Regulation of Macrophage Responses

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We studied the role of nitric oxide in the stress response and apoptosis. Intracellular nitric oxide potentiated the stress response. However, intracellular nitric oxide suppressed the stress response in macrophages of proinflammatory and antiinflammatory phenotypes. Intracellular nitric oxide promoted apoptosis in macrophages of the proinflammatory phenotype, but inhibited this process in cells of the antiinflammatory phenotype. Exogenous nitric oxide synthesized by macrophages protected them from lipopolysaccharide-induced apoptosis. Our results indicate that nitric oxide produces various effects on the stress response and apoptosis in macrophages, which depends on *modus operandi*.

Key Words: *stress response; apoptosis; macrophages; nitric oxide*

Macrophages play an important signal and regulatory role in immune reactions of the organism. Macrophage-derived cytokines provide innate and adaptive immunity.

Secretory activity of macrophages varies thus maintaining the immune balance. Exposure of macrophages to lipopolysaccharide (LPS) in various concentrations modifies the naive pattern of secreted cytokines into the proinflammatory or antiinflammatory pattern. During further stimulation of these macrophages with various pathogenic products (LPS in various concentrations, yeast zymosan A, and gram-positive *S. aureus*), the antiinflammatory phenotype produces a greater amount of antiinflammatory cytokines interleukin-10 (IL-10) and nitric oxide (NO) and lower amount of proinflammatory cytokines IL-6, IL-12, and tumor necrosis factor-α compared to the proinflammatory phenotype [7]. Studying the phenomenon of macrophage reprogramming (LPS-dependent alternative acquisition of a specific phenotype) formed the basis for the general concept of macrophage-dependent regulatory mechanisms of the innate and acquired immune response to microbial pathogen invasion [7].

The stress response is a protective mechanism activated by macrophages and counteracting the toxic effect of proinflammatory cytokines and oxidative and nitric oxide stress [4,5]. Inducible heat shock proteins HSP70 play a major role in the stress response [2]. However, prolonged activation of macrophages and overproduction of inflammatory mediators initiate a variety of pathological processes and contribute to the developments of diseases. The approach to limit severe inflammatory response suggests stimulation of apoptosis in activated macrophages. A balance between the stress and apoptotic responses determines the behavior of activated macrophages (preservation or death of cells in the inflammatory site).

The stress response and apoptosis in macrophages are regulated by NO. SH groups in proteins [6] and iron-containing proteins are the targets for NO [3]. NO induces direct modification of proteins via nitrosylation or has an indirect effect on me-

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thylation and ribosylation. NO synthesis in the cytoplasm of macrophages is catalyzed by iNOS. NO crosses the cell membranes and interacts with various intracellular and extracellular biological structures. NO regulates activity of cytoplasmic guanylate cyclase and inhibits iNOS and several mitochondrial enzymes. Extracellular NO is probably involved in nitrosylation of SH groups in extracellular domains of membrane receptors and, therefore, plays a role in specific signal pathways. It can be hypothesized that the effect of NO on the stress response and apoptosis depends on the primary target for this compound (extra- or intracellular target).

In the present work this hypothesis was tested on macrophages of proinflammatory and antiinflammatory phenotypes.

MATERIALS AND METHODS

Experiments were performed on cultured mouse macrophages. Proinflammatory and antiinflammatory phenotypes of macrophages were obtained by the method of Morrison [7]. The primary culture of naive macrophages was divided into 3 equal pools. LPS was added to pools I (0.5 ng/ml) and II (5 ng/ml) to induce proinflammatory and antiinflammatory phenotypes, respectively. Pool III served as the control. The stress response and apoptosis were induced by LPS in a concentration of 500 ng/ml. The stress response was evaluated by HSP70 content (Western blot analysis). Apoptosis was assayed by DNA fragmentation (flow cytometry).

The intracellular and extracellular regulatory effects of NO on the stress response and apoptosis in LPS-stimulated macrophages were studied using ITU (S-(2-aminoethyl)isothiourea) and c-PTIO ((2-4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxil-3-oxide). ITU serves as a selective iNOS inhibitor. c-PTIO is a NO trap that does not cross the cell membrane and cannot enter the cell [1].

The results were analyzed by Student's *t* test. The data are presented as $M \pm m$.

RESULTS

Figure 1 illustrates the effect of iNOS inhibition and extracellular NO binding on the stress response in macrophages of various phenotypes (Fig. 1). Combined treatment with iNOS inhibitor ITU and LPS in a concentration of 500 ng/ml completely blocked LPS-induced activation of HSP70 synthesis in macrophages of various phenotypes. c-PTIO was added to neutralize extracellular NO. This agent potentiated LPS-induced synthesis of HSP70.

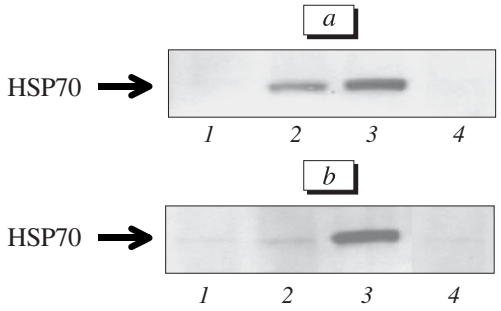

Fig. 1. Effects of ITU and c-PTIO on LPS-induced HSP70 synthesis in macrophages of proinflammatory (*a*) and antiinflammatory phenotypes (*b*). Control (*1*); LPS (*2*); LPS+c-PTIO (*3*); LPS+ITU (*4*).

NO synthesized by macrophages provides cytotoxicity relative to pathogenic bacteria. NO should be rapidly eliminated from macrophages into the extracellular space. The intensity of NO production in antiinflammatory macrophages is higher than in proinflammatory macrophages [7]. NO concentration is high around antiinflammatory macrophages, which prevents the LPS-induced stress response. This specific feature probably determines suppression of the stress response in macrophages of the antiinflammatory phenotype.

We studied the role of NO in modulation of LPS-induced apoptosis in macrophages of various phenotypes (Table 1). LPS-induced apoptosis 2-fold decreased in cells of proinflammatory phenotype, but 4-fold increased in cells of antiinflammatory phenotype after treatment with iNOS inhibitor ITU. Our results indicate that intracellular NO produced by iNOS plays a proapoptotic role in cells of proinflammatory phenotype, but produces the antiapoptotic effect on cells of antiinflammatory phenotype.

c-PTIO increases apoptosis in cells of various phenotypes. Hence, exogenous NO synthesized by macrophages protects them from LPS-induced apoptosis. It cannot be excluded that this mechanism determines the autocrine protection of macrophages in the inflammatory site.

Our results indicate that NO produces various effects on the stress response and apoptosis in ma-

TABLE 1. Effects of ITU and c-PTIO on LPS-Induced DNA Fragmentation in Macrophages of Proinflammatory and Antiinflammatory Phenotypes ($M \pm m$)

Phenotype	LPS	LPS+c-PTIO	LPS+ITU
Proinflammatory	11±2	20±2*	32±3*
Antiinflammatory	8±1	2±1*	3±1*

Note. Percentage of cells with defragmented DNA. *$p<0.05$ compared to LPS.

crophages, which depends on the target for this compound (extra- or intracellular target).

The drugs that affect various pools of NO selectively modulate the stress and apoptotic responses in macrophages of various phenotypes. These data hold promise to develop new immunotherapeutic methods for the therapy of diseases associated with inflammation (sepsis, atherosclerosis, diabetes, and ischemic injury of the brain and heart).

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