Inversion of Stress Response Reprogramming Phenomenon in Lipopolysaccharide-Stimulated Alveolar Macrophages

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The stress response and NO production in reprogrammed proinflammatory or antiinflammatory alveolar macrophages were studied after lipopolysaccharide treatment. Experiments with macrophages not containing HSP70 showed that lipopolysaccharide in a dose of 500 ng/ml induced stress response in cells with the proinflammatory phenotype (as distinct from an antiinflammatory phenotype). The stress response was not observed in HSP70-containing lipopolysaccharide-stimulated proinflammatory macrophages, but occurred in cells with antiinflammatory phenotype. Hence, the presence of HSP70 in alveolar macrophages results in the inversion of the phenomenon of reprogramming of the stress response. Independently on the phenotype, stimulation with lipopolysaccharide was accompanied by a 60-70% increase in NO production by macrophages not containing HSP70. However, NO production by HSP70-containing macrophages did not increase in response to lipopolysaccharide treatment. Our results indicate that reprogramming of the cell response in macrophages does not concern the system for NO synthesis. HSP70 prevents the lipopolysaccharide-induced activation of NO synthesis in alveolar macrophages.

Key Words: *stress response; alveolar macrophages; lipopolysaccharides; NO; reprogramming*

Inflammatory reactions of the organism play an important role in the development of various lung diseases, including asthma, acute respiratory distress syndrome, and bronchopulmonary dysplasia. Evaluation of the molecular mechanisms and modulation of inflammatory response in the lungs are urgent problems of modern medicine.

Macrophages play a key role in the induction and development of lung inflammation. Activated macrophages produce free radicals, NO, cytokines, chemokines, and other inflammatory mediators. Hence, these cells initiate the early inherited immune response that is directed toward elimination of microbial pathogenic products. Macrophages provide the regulatory signals for differentiation of T cells and development of the cellular or humoral adaptive immune response [5,11]. During the immune response, native macrophages gain proinflammatory (PIP) or antiinflammatory phenotype (AIP). PIP of activated macrophages is characterized by production of proinflammatory cytokines, including interleukin-1 (IL-1), IL-12, and tumor necrosis factor-α. AIP determines reduced production of proinflammatory cytokines and increased synthesis of antiinflammatory cytokines (*e.g.*, IL-10) [13]. Transformation of macrophage phenotype received the name reprogramming [13].

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Macrophages are exposed to the influence of adverse factors in the inflammatory focus. Activation of self-protecting stress response in macrophages prevents the toxic effect of proinflammatory cytokines and oxidative and nitrosative stress [8,9]. Inducible heat shock proteins HSP70 play a key role in this process [3]. However, activation of HSP70 synthesis and specific features of the protective stress response in alveolar macrophages of various phenotypes remain unknown. Various cytokines have different effects on HSP70 synthesis [6,10,12]. Moreover, macrophages of various phenotypes produce different cytokines (proinflammatory or antiinflammatory agents). It may be suggested that the stress response in alveolar macrophages depends on the phenotype. Experiments with mouse peritoneal macrophages suggest that NO plays a key regulatory role in HSP70 synthesis by macrophages [2].

The stress response provides survival of activated macrophages under adverse conditions. Hence, phenotype-dependent activation of the stress response can be associated with the period of functional activity of activated PIP and AIP macrophages and production of proinflammatory and antiinflammatory mediators in the lungs. Study of these problems will elucidate the pathogenesis of lung diseases and allow the development of new approaches to the therapy for lung inflammation.

Here we tested the hypothesis on phenotype-dependent stress response in alveolar PIP and AIP macrophages. The role of NO in phenotype-dependent activation of the stress response was evaluated.

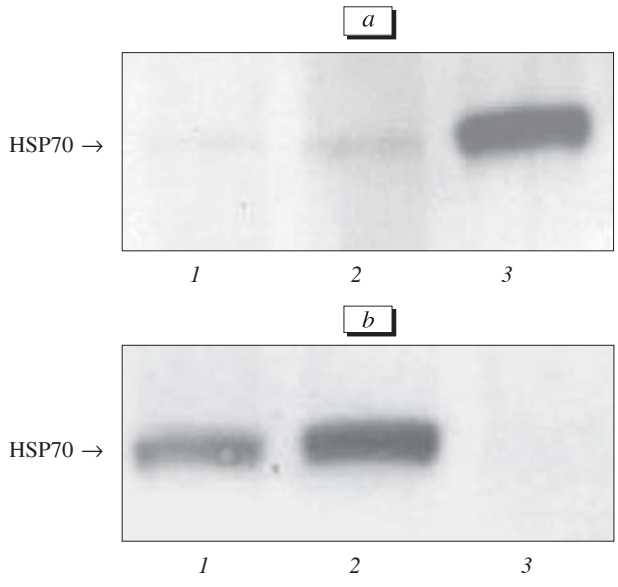

Fig 1. LPS-induced stress response in HSP70$^-$ (*a*) and HSP70$^+$ (*b*) PIP and AIP alveolar macrophages. Here and in Fig. 2: native macrophages before reprogramming (*1*); AIP macrophages 24 h after stimulation with LPS (*2*); and PIP macrophages 24 h after stimulation with LPS (*3*).

MATERIALS AND METHODS

Alveolar macrophages were isolated from the bronchial alveolar fluid of Wistar rats. The primary culture of native alveolar macrophages was divided into 3 pools to obtain PIP and AIP macrophages [13]. Lipopolysaccharide (LPS) was added to pools I (0.5 ng/ml) and II (5 ng/ml) for 6 h to induce PIP and AIP, respectively. Pool III served as the control. The stress response and NO synthesis were induced by LPS in a concentration of 500 ng/ml. The stress response was evaluated from HSP70 content (Western blotting). NO production by macrophages was estimated spectrophotometrically in the Griess reaction. Nitrite concentration in the culture medium was measured.

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

RESULTS

The population of Wistar rats is not genetically homogeneous. This specific feature determines several differences in the response of macrophages from rats of various litters of the population. Our study involved animals of various litters, which were bred from different families. Alveolar macrophages from various litters of Wistar population differed in the basal content of HSP70 (Fig. 1). HSP70 were not identified in macrophages from rats of one litter, but were present in macrophages from animals of another litter. The cell culture of intact and unstimulated macrophages not containing HSP70 was designated as HSP70$^-$. Macrophages isolated from rats of another litter and containing HSP70 were designated as HSP70$^+$. The presence of HSP70$^-$ and HSP70$^+$ alveolar macrophages in Wistar rats of various litters allowed us to evaluate the dependence of macrophage reprogramming on the basal content of HSP70.

Pools of HSP70$^-$ and HSP70$^+$ macrophages were reprogrammed for PIP and AIP [13]. In HSP70$^-$ macrophages, LPS in a dose of 500 ng/ml induced the stress response of native and PIP macrophages, but had little effect on AIP macrophages (Fig. 1). Therefore, activation of the stress response in alveolar macrophages is a phenotype-dependent process. The data on rat alveolar HSP70$^-$ macrophages are consistent with the results of experiments on mouse peritoneal macrophages. It was shown that native cells do not contain HSP70 [1]. Hence, reprogramming of the stress response is not a species-specific reaction (rats or mice). Moreover, this

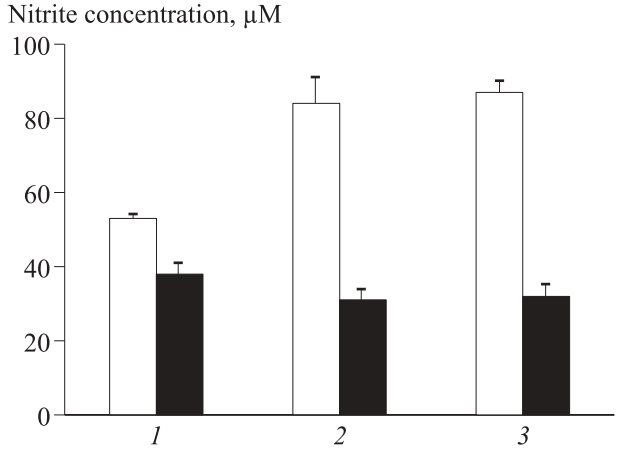

Fig. 2. LPS-induced production of NO in HSP70$^-$ (light bars) and HSP70$^+$ (dark bars) PIP and AIP alveolar macrophages.

phenomenon does not depend on the source of macrophages (peritoneal or alveolar cells).

HSP70 synthesis did not increase in LPS-stimulated HSP70$^+$ macrophages with native phenotype and in PIP macrophages. Hence, the stress response did not occur in these cells. However, the stress response was observed in AIP macrophages (Fig. 1). Our results indicate that activation of the stress response in alveolar macrophages is determined by the phenotype (independently on the basal content of HSP70). The presence of HSP70 in alveolar macrophages causes inversion of the phenomenon of reprogramming of the stress response. Experiments with macrophages not containing HSP70 showed that reprogramming results in the induction of the stress response in macrophages with native phenotype and in PIP macrophages after LPS treatment. After reprogramming, LPS induced the stress response only in HSP70-containing AIP macrophages. Therefore, reprogramming of the stress response of alveolar macrophages to bacterial factors can be regulated by modulation of intracellular HSP70.

There are conflicting data on the LPS-induced stress response. Some authors revealed induction of HSP70 in LPS-stimulated macrophages [7]. Other authors reported that stimulation of macrophages with LPS was not accompanied by activation of HSP70 synthesis [4]. Cytokines and NO play a role in modulation of HSP70 synthesis, which provides new data on regulation of the LPS-induced stress response. Differentially reprogrammed macrophages are phenotypically characterized by the unique set of secreted cytokines and other mediators of inflammation. We hypothesized that the LPS-induced stress response in alveolar macrophages is a phenotype-dependent process. The results of our study provide support for this hypothesis. We found that HSP70 prevents the LPS-induced stress response in native and PIP macrophages. Our results extend our knowledge on the regulation of LPS-induced stress response. Treatment of macrophages with LPS triggers the negative feedback mechanism, which regulates the stress response. Moreover, our study explains the discrepancy between the results obtained in various laboratories. Such discrepancy results from the fact that these experiments were performed on macrophages differing in the phenotype or basal level of HSP70.

Basal production of NO in HSP70$^-$ macrophages was much higher than in HSP70$^+$ macrophages. Independently on the phenotype, NO production in HSP70$^-$ macrophages increased by 60-70% after stimulation with LPS. However, NO production in HSP70$^+$ macrophages did not increase in response to LPS treatment (Fig. 2).

Our results indicate that reprogramming of the cell response in macrophages does not concern the system for NO synthesis. The effect of LPS on NO synthesis in alveolar macrophages does not depend on the phenotype of macrophages. It may be suggested that NO does not play a role in phenotype-dependent activation of the stress response in alveolar macrophages. However, intracellular and extracellular NO produces various effects on the stress response [2]. These effects may compensate for each other. Studies with intracellular inhibitors of NO synthesis and extracellular NO-trapping agent should be performed to evaluate the role of NO in phenotype-dependent activation of the stress response.

Intracellular HSP70 suppresses the basal and LPS-induced activation of NO synthesis in alveolar macrophages. Hence, NO production (one of the key functions of macrophages) can be modulated by affecting the basal level of HSP70 in alveolar macrophages. Independently on the induction of HSP70 synthesis (positive effect in PIP and native macrophages, negative effect in AIP macrophages) LPS treatment is followed by NO production in LPS-activated HSP70$^-$ macrophages. This process did not differ in cells of various phenotypes. These data suggest that HSP70 prevents induction of NO synthesis, but has no effect on the previously initiated process.

Published data show that NO regulates HSP70 synthesis and stress response in cells [2]. We revealed that HSP70 may prevent the induction of NO synthesis in alveolar macrophages. Hence, the immune response in macrophages includes a biological autoregulatory circuit NO—HSP70.

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