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Effect of Oxidized Dextrans on NO Synthase and Arginase Activities of Mouse Macrophages

V. O. Tkachev, O. P. Kolesnikova*, A. V. Troitskii, and V. A. Shkurupii

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We studied the effect of oxidized dextrans with molecular weights of 30-35 kDa and 60-65 kDa on NO synthase and arginase activities of mouse peritoneal macrophages *in vivo* and *in vitro*. Oxidized dextrans irrespective of molecular weight shifted the NO synthase/arginase balance towards predominance of NO production under *in vivo* and *in vitro* conditions. Administration of the test compounds to intact mice considerably increased NO synthase activity, while culturing of peritoneal macrophages in the presence of modified dextrans reduced arginase activity in these cells. These effects of oxidized dextrans create conditions for predominant stimulation of Th1-mediated immune reactions.

Key Words: *oxidized dextrans; macrophages; nitric oxide; arginase*

Targeted delivery of bioactive compounds is an actual problem of modern pharmacology, which can be realized by using high-molecular-weight biocompatible and biodegradable carriers of pharmacological drugs modulating functions of immunocompetent cells, in particular, mononuclear phagocyte system (MPS) cells.

These carriers, *e.g.* oxidized dextrans, are effectively absorbed by macrophages and are hydrolyzed by lysosomal enzymes [2], which is accompanied by various changes in macrophage metabolism.

It was shown that the proportion between alternative pathways of arginine metabolism (NO synthase and arginase) largely determines the capacity of macrophages to stimulate cell-mediated (Th1-dependent) or humoral (Th2-dependent) immune reaction [6,7]. Thus, substances shifting the NO synthase/arginase balance can be used for modulation of the immune response and correction of its disturbances. Increased production of NO due to activation of inducible NO synthase is a key element in the pathogenesis of ulcerative colitis induced by sulfated dextrans [4,9] and in the formation of dextran-induced lung granulomas [11]. According to other studies, phagocytosis of sulfated dextrans *in vivo* leads to inhibition of NO synthase [5]. The effects of dextrans on macrophage arginase was not studied.

Here we studied the effect of oxidized dextrans on arginase and NO synthase activity of mouse macrophages.

MATERIALS AND METHODS

Experiments were performed on 2-month-old female $(C57Bl/6\times DBA/2)F_1$ mice weighing 20-22 g (nursery of Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences).

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences; *Research Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** tkachev_victor@gorodok.net. V. O. Tkachev

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In *in vivo* experiments, chemically oxidized dextrans [3] with molecular weights of 30-35 kDa and 60-65 kDa were injected intraperitoneally in a dose of 50 mg/mouse (10% solutions in 0.85% NaCl) 24 and 48 h before isolation of macrophages. In *in vitro* experiments, oxidized dextrans were added to culture media and incubated with macrophages in concentrations of 0.25 and 0.16 g/liter.

The mice were sacrificed by cervical dislocation under ether narcosis. Cold RPMI-1640 medium (10 ml) containing 1% fetal calf serum (FCS) was injected into the peritoneal cavity and after 2 min this culture medium containing peritoneal exudation cells was collected with a syringe. The cell suspension was centrifuged 10 min at 1.5×10^3 rpm. The pellet was resuspended in complete culture medium prepared on the basis of RPMI-1640 (without phenol red) supplemented with 10% FCS, 15 mM HEPES, and 0.3% L-glutamine. The cells were seeded to 96-well plates (200×10^3 cells in 200 μl per well). After 2-h incubation (37°C, 5% CO_2), nonadherent cells were removed by 2-fold washout with warm RPMI-1640 medium. The adherent fraction of peritoneal exudate cells contained primarily macrophage cells identified by morphological features.

The intensity of NO production was evaluated by the content of nitrites (in μM) in supernatants of cell cultures. NO production was stimulated by adding LPS (*E. coli* B5:055, 10 mg/ml) and LPS in combination with IFN-γ to macrophage cultures. Supernatant of mixed lymphocyte culture obtained by standard method and containing IFN-γ in a concentration of 1 U/ml served as the source of IFN. After 48-h culturing, 100 μl supernatant was taken from each well and mixed 1:1 with Griess reagent in wells of a 96-well flat-bottom plate for immunological reactions. The plates were incubated in darkness for 15 min and then optical density at λ=540 nm was measured.

Arginase activity was determined by the rate of urea formation. Macrophages in wells of a culture plate were lyzed with 0.1% Triton X-100 and 50 μl lysate was mixed with 50 μl 50 mM Tris-HCl (pH 7.4) and 10 μl 50 mM manganese chloride. Arginase was activated by heating on a water bath at 57°C for 10 min, then 100 μl 0.5M L-arginine was added, and the mixture was incubated for 30 min at 37°C. The reaction was stopped by adding $H_2SO_4/H_3PO_4/H_2O$ (1:3:7, v/v/v). The concentration of urea was measured colorimatrically (at λ=540 nm) after adding a-nitrosopropiophenone (9% ethanol solution) and 30-min heating on a water bath.

Macrophages isolated from intact animals were used as the control for measurement of NO-synthase and arginase activities.

The data were processed statistically using nonparametric Mann—Whitney test (Statistica 6.0 software).

RESULTS

Culturing in the presence of oxidized dextran with a molecular weight of 30-35 kDa increased LPS-stimulated NO production by macrophages (at a final dextran concentration of 0.25 g/liter, Fig. 1) and had no effect on LPS/IFN-γ-stimulated NO production (Fig. 2). Oxidized dextran with a molecular weight of 60-65 kDa *in vitro* had no effect on LPS-stimulated activity of NO-synthase in macrophages and suppressed NO production stimulated by LPS in combination with IFN-γ (Fig. 1, 2). At the same time, administration of oxidized dextrans to intact animals 24 and 48 h before cell isolation considerably increased NO production stimulated by LPS alone and in combination with IFN-γ (Fig. 1,2) irrespective of the molecular weight of the administered dextrans.

Thus, *in vivo* and *in vitro* effects of oxidized dextrans on the NO synthase system in macrophages considerably differed. This can reflect the important role of cell-cell cooperation for activation of NO synthesis mediated by modified dextrans; T lymphocytes synthesizing proinflammatory cytokines can be the major partner of macrophages in this process [8]. The decrease in LPS/IFN-γ-induced NO production observed *in vitro* can be explained by activation of superoxide-producing system, one of the most important physiological regulators of NO production, in macrophages [10].

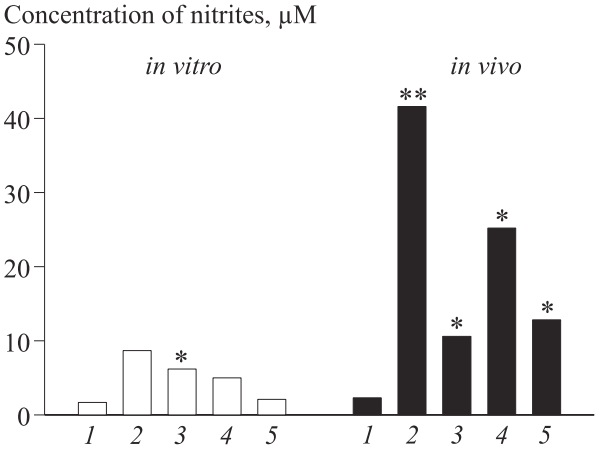

Fig. 1. Effect of oxidized dextrans on LPS-stimulated NO production by macrophages. Here and on Fig. 2, 3: *in vitro*: *1*) control; *2*) dextran 30 kDa, 0.16 g/liter; *3*) dextran 30 kDa, 0.25 g/liter; *4*) dextran 60 kDa, 0.16 g/liter; *5*) dextran 60 kDa, 0.25 g/liter. *in vitro*: *1*) control; *2*) dextran 30 kDa, 24 h; *3*) dextran 30 kDa, 48 h; *4*) dextran 60 kDa, 24 h; *5*) dextran 60 kDa, 48 h. *p<0.01, **p<0.05 compared to the control.

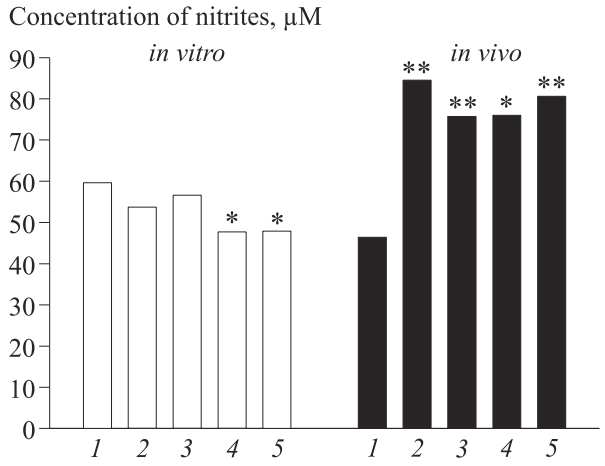

Fig. 2. Effect of oxidized dextrans on LPS/IFN-γ-stimulated NO production by macrophages.

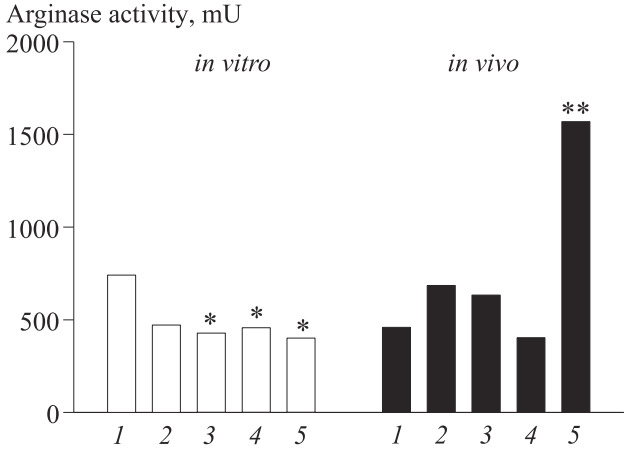

Fig. 3. Effect of oxidized dextrans on arginase activity in macrophages. The amount of enzyme synthesizing 1 mmol urea per min was taken as 1 U.

Administration of modified dextran with a molecular weight of 60-65 kDa 48 h before macrophage isolation increased arginase activity in cells (Fig. 3), but to a lesser extent compared to activation of NO synthase. It can be hypothesized that physiological role of stimulation of arginase activity observed in this case consists in prevention of excessive NO production, because high concentrations of NO can produce damaging effects on cells.

Oxidized dextrans under *in vitro* conditions suppress arginase activity in macrophages (Fig. 3), which creates prerequisites for activation of the NO synthase system.

Thus, our findings suggest that oxidized dextrans with various molecular weight can considerably shift the balance between NO synthase and arginase in macrophages. On the whole, predominance of NO synthase activity promotes polarization of macrophages towards M-1 producing proinflammatory cytokines and stimulating cell-mediated immune reactions. The described effects of oxidized dextrans on arginine metabolism in macrophages suggest that the test compounds can be used as biocompatible matrix carriers of bioactive substances, including drugs with antibacterial and antifungal properties for their targeted delivery and modulation of functions of macrophages and MPC cells on the whole.

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