Effect of Lipopolysaccharides of Various Composition from Gram-Negative Bacteria on Macrophage Activity, Oxidant Metabolism, and Microsomal Activity of the Liver

S. V. Sibiryak, S. A. Sergeeva, T. G. Khlopushina, N. N. Kurchatova, and R. Sh. Yusupova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 183-186, February, 1998
Original article submitted December 2, 1996

The study explores the effect of lipopolysaccharides of various composition from Gram-negative bacteria on phagocytic activity and oxygen-dependent metabolism of macrophages, intensity of lipid peroxidation, and activity of microsomal enzymes in mouse liver, and production of interleukin-1 and tumor necrosis factor by cultured peripheral blood mononuclears. It is found that the magnitude of inhibiting effect of lipopolysaccharides on microsomal oxidation is a mirror reflection of the degree of their prooxidant effect and activation of phagocytic and secretory macrophage functions, which confirms tight coupling of these processes under conditions of acute phase response.

Key Words: *bacterial lipopolysaccharides; microsomal oxidation; macrophage activity*

It has been found that administration of various immunostimulators and the development of viral and bacterial infections are accompanied by suppression of cytochrome P-450-dependent liver monooxygenases in hepatic and other tissues. This effect is related to macrophage activation and is mediated by interferon and early cytokines such as interleukin-1, interleukin-6, tumor necrosis factor (TNF) etc., which inhibit the cytochrome P-450 gene expression [4,13, 14]. Inhibition of microsomal oxygenases is probably required for glucocorticoid biotransformation under conditions of acute phase reaction and in cooperation with other factors promotes restoration of homeostasis in immune stress [5].

On the other hand, generation of reactive oxygen species, oxidized halogenides, and nitric oxide by macrophages, activation of lipid peroxidation (LPO), and a shift of oxidant balance are other important components of acute phase reaction [8]. Induction of free radial processes results in modulation of physicochemical properties of biological membranes and suppression of cytochrome P-450-dependent monooxygenases [12].

These processes are tightly coupled, the degree of macrophage activation being the key factor determining their activity. For verification of this assumption we used different lipopolysaccharides (LPS) from Gram-negative bacteria. These LPS produce the same immunotropic effects but are characterized by different activity and toxicity, which depend on the content of their protein components, lipid A, and various monosaccharides in polysaccharide complexes [2].

MATERIALS AND METHODS

The study was carried out on random-bred male mice weighing 18-20 g. Effect of immunostimulators on cytokine production was assessed using a culture

Department of Immunology and Immunopharmacology, Russian Center of Eye and Plastic Surgery, Ufa, Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

0007-4888/98/0002-0159$20.00

of peripheral blood macrophages obtained from healthy donors.

LPS-protein complexes (whole endotoxins, LPS-PC) and protein free LPS (protein content less than 1%) of mutant *Escherichia coli* Mre-600 and *Shigella sonnei* 90-90 strain were used. LPS and LPS-PC were isolated, purified, and chemically verified at the Laboratory of Subcellular Bacterial Structures (G. N. Gabrichevskii Institute of Microbiology and Epidemiology, Ministry of Health). The commercial immunostimulator prodigiozan, LPS of *Serratia marcescens* (Moskhimfarmpreparat), was also used.

Phagocytic activity of the reticuloendothelial system, Kupffer cells, and spleen macrophages was assessed spectrophotometrically (650 nm) by elimination rate for intravenously injected colloid carbon (160 mg/kg). The data were presented as corrected phagocytic index [10].

The intensity of respiratory burst in peritoneal macrophages was assessed by luminol-dependent chemiluminescence in the presence of 10^{-6} luminol. The intensity of LPO was evaluated by measuring integral intensity of slow flash of Fe^{2+}-induced chemiluminescence in liver homogenate [6] in the incubation medium containing 20 mM KH_2PO_4, 105 mM KCl, 2.5 mM Fe^{2+} (final concentration), pH 7.45, at 37°C and constant stirring (800 rpm). This medium ensures maximum catalytic activity of Fe^{2+}. All measurements were carried out in a KhL-003 chemiluminometer (Soyuz).

Microsomes were isolated by differential centrifugation [7]. The contents of cytochrome P-450 [11] and aminopyrine N-demethylase, aniline p-hydroxylase activities [3], and protein concentration in the microsomal fraction were measured [9].

The concentrations of interleukin-1 and TN were measured in supernatants of peripheral blood mononuclear cultures obtained from healthy donors after a 4-h incubation in the presence of LPS and LPS-PC (5 μg/ml) using ProCon IL-1β and ProCon TNF-α enzyme-linked immunosorbent test systems (Institute of Extrapure Preparations, St. Petersburg) Mononuclear cells were routinely isolated in a Ficoll-Isopaque gradient (Flow Lab.).

The data were processed statistically using the Student *t* test.

RESULTS

Intraperitoneal injection of the immunostimulators in a dose of 5 μg/mouse one day prior to testing considerably enhanced phagocytic activity of the reticuloendothelial system, which manifested itself as accelerated elimination of intravenously injected carbon from circulation and increased corrected phagocytic index (Table 1). It should be noted that Kupffer cells and spleen macrophages execute 90% elimination function of the reticuloendothelial system. All test LPS and LPS-PC induced potent respiratory burst in phagocytes: the intensity of luminol-dependent chemiluminescence of stimulated macrophages 100-400% surpassed the control level. Maximum stimulation of phagocytosis and metabolism was achieved with prodigiozan and LPS-PC from *E.coli*, LPS-PC from *S.sonnei* was much less active, purified LPS from *S.sonnei* and *E. coli* produced an intermediate effect. Generation of reactive oxygen species was accompanied by LPO activation in the microenvironment and accumulation of hydroperoxides interacting with Fe^{2+} in the model system. Integral intensity of

TABLE 1. Effects of Different Lipopolysaccharides from Gram-Negative Bacteria on Phagocytic Activity of Reticuloendothelial System, Generation of Reactive Oxygen Species by Peritoneal Macrophages, and Fe^{2+}-Induced Chemiluminescence in Mouse Liver Homogenate (*M±m*)

Group	Phagocytic activity, corrected phagocytic index (*n*=6)	Oxidant metabolism (*n*=5)	
		luminol-dependent chemiluminescence of peritoneal macrophages, integral intensity/ 10^3 cells/3 min, rel. units	Fe^{2+}-induced chemiluminescence of liver homogenate, integral intensity of slow flash over 5 min, rel. units
Control	6.02±0.56	3.0±0.4	77.0±4.1
LPS from *E. coli*	7.42±10.30*	7.3±2.2*	82.0±9.8
LPS-PC from *E. coli*	10.1±0.80*	12.6±2.9*	104.0±8.6*
LPS from *S. sonnei*	9.00±0.80*	8.4c1.6*	93.0±6.8
LPS-PC from *S. sonnei*	9.03±0.40*	11.4±1.6*	97.0±9.7
Prodigiozan	9.94±0.35*	12.4c1.4*	105.0±10.3*

Note. Here and in Tables 2 and 3: *n* is the number of mice, *$p<0.05$ compared with the control.

slow flash of Fe^{2+}-induced chemiluminescence in liver homogenates from LPS-treated mice was increased and reflected the differences in activity between the test preparations. However, despite the development of substantial respiratory burst in macrophages, activation of LPO was much less pronounced: the differences were significant only in mice treated with prodigiozan and whole *E.coli* endotoxin. Dramatic changes were impossible, since induction of free radical processes is always accompanied by activation of tissue antioxidant systems.

Test LPS had different effects on production of early cytokines such as interleukin-1 and TNF by cultured mononuclears (Table 2). Prodigiozan and whole *E.coli* and *S. sonnei* endotoxins were most effective, while protein-free LPS from *E.coli* and *S. sonnei* induced only an insignificant increase in the concentration of these cytokines in supernatants.

Liver microsomes were isolated one day after intraperitoneal injection of LPS and LPS-PC. The effect of these immunostimulators on the monooxygenase system was a mirror reflection of their effect on macrophage activity and the intensity of LPO in the liver (Table 3). The most pronounced decrease in the contents of cytochrome P-450 and aminopyrine N-demethylase and in aniline p-hydroxylase activities was noted in mice treated with prodigiozan and LPS-PC from *E.coli* and *S. sonnei*, while in animals injected with purified LPS, significant changes were observed only for aminopyrine N-demethylase activity. It should be noted that bacterial LPS do not form enzyme-substrate complex with cytochrome P-450 and are metabolized in phagocytes.

Our findings suggest that the inhibiting effect of different LPS from Gram-negative bacteria on the system of cytochrome P-450-dependent monooxygenases is proportional to their stimulating effect on macrophages (phagocytic function, generation of reactive oxygen species, secretion of interleukin-1 and TNF) and accumulation of LPO products in the liver.

TABLE 2. Effect of LPS from Gram-Negative Bacteria on Cytokine Production by Cultured Human Mononuclears (4-h Incubation, *M±m*)

Group	Cytokine production, pg/ml	
	interleukin-1β	tumor necrosis factor-α
Control	148.4±16.0	66.6±3.2
LPS from *E.coli*	148.4±116.0	66.6±3.2
LPS-PC from *E.coli*	273.0±52.4*	95.9±10.8*
LPS from *S. sonnei*	194.4±20.8	74.9±4.4
LPS-PC from *S. sonnei*	233.2±2.4*	118.7±6.8*
Prodigiozan	255.0±21.4*	91.6±2.2*

Thus, it can be assumed that under conditions of acute phase reaction and macrophage activation, regulation of microsomal oxidation is effected through both distant (cytokine-mediated) and local (shifts in oxidant balance) mechanisms. This is consistent with the concept on the stress-realizing role of free radical processes in the organism [1] and provides new approaches to pharmacological correction of acute phase response and endotoxic reactions.

We are grateful to Drs. T. N. Belkin and T. P. Egorova for providing LPS and endotoxins.

REFERENCES

1. V. A. Baraboi, I. I. Brekhman, V. G. Golotin, and Yu. B. Kudryashov, *Lipid Peroxidation and Stress* [in Russian], St.Petersburg (1992).
2. Z. V. Ermol'eva, *Antibiotics, Interferon, and Bacterial Lipooly-saccharides* [in Russian], Moscow (1968).
3. I. I. Karuzina and A. I. Archakov, in: *Modern Methods in Biochemistry* [in Russian], Moscow (1977).
4. S. V. Sibiryak, I. L. Krasilova, L. A. Ryabchinskaya, *et al.*, *Farmakol. Toksikol.*, No. 4, 46-48 (1992).
5. S. V. Sibiryak, S. A. Sergeeva, and I. L. Krasilova: *Neuroimmunology. Neuroinfections. Neuroimage* [in Russian], St.Petersburg (1995), pp. 106-109.

TABLE 3. Effect of LPS from Gram-Negative Bacteria on the Content of Cytochrome P-450 and Monooxygenase Activity in Mouse Liver Microsomes (*M±m*)

Group	Content of cytochrom P-450, nmol/mg protein (*n*=7)	Enzyme activity, nmol/min/mg protein	
		aminopyrine N-demethylase	aniline p-hydroxylase
Control	0.30±0.01	9.05±0.12	1.06±0.02
LPS from *E.coli*	0.28±0.01	6.43±0.29*	0.83±0.07
LPS-PC from *E.coli*	0.21±0.03*	6.07±0.26*	0.76±0.03*
LPS from *S. sonnei*	0.28±0.01	6.68±0.42*	0.86±0.04
LPS-PC from *S. sonnei*	0.24±0.01*	6.03±0.56*	0.67±0.08*
Prodigiozan	0.21±0.02*	5.20±0.40*	0.55±0.12*

6. R. R. Farkhutdinov and V. A. Likhovskikh, *Chemiluminescent Methods in the Study of Free Radical Processes in Biology and Medicine* [in Russian], Ufa (1995).
7. J. Ashokas, O. Pelkonen, and N. Karki, *Cancer Res.*, **37**, 3737-3743 (1977).
8. H. Baumann and J. Gauldi, *Immunol. Today*, **15**, 74-81 (1994).
9. E. Hartee, *Anal. Biochem.*, **48**, 422-427 (1972).
10. L. Hudson and F. Hay, *Practical Immunology*, 2nd ed. London (1980).
11. T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370-2378 (1964).
12. D. Parke, C. Ioannides, and D. Lewis, *Can. J. Physiol. Pharmacol.*, **69**, 537-539 (1991).
13. K. Sujita, F. Okuno, Y. Tanaka, *et al.*, *Biochem. Biophys. Res. Commun.*, **168**, 1217-1222 (1990).
14. K. Wright and E. Morgan., *Mol. Pharmacol.*, **39**, 468-474 (1991).

Changes of α-B-Crystalline in Cardiac Grafts

L. I. Kovalev*, E. S. Ershova*, L. I. Obel'chuk, D. A. Tsomartova, P. Z. Khasigov, V. V. Severin, E. I. Ryaboshtanova**, I. M. Il'inskii**, and S. S. Shishkin***

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 187-189, February, 1998
Original article submitted January 22, 1997

Polypeptide composition of cardiac grafts is studied using two-dimensional electrophoresis. Changes in the content of α-chain of B-crystalline were found, which correlated with remoteness of chronic cardiac graft rejection. In the kidney of patient with chronic cardiac graft rejection, unusual concentrations of α-B-crystalline and products of its degradation (microsequence data) were found.

Key Words: *transplantation; crystalline; man; two-dimensional electrophoresis*

Many problems of practical transplantology and delayed outcomes of heart transplantation are related to the development of chronic graft rejection. Diagnosis of chronic graft rejection is based primarily on clinical manifestations and physiological and biochemical tests. Morphological analysis provides the most precise diagnosis [1,5]. Molecular processes remain poorly studied; however, molecular markers of pathological processes are the most accurate and early diagnostic signs.

Extensive systemic studies of protein spectra with the use of two-dimensional electrophoresis (TDE) and other precise methods [2,8,9] open new prospects in the investigation of molecular mechanisms and screening for marker proteins of various complications in organ transplantation. In the future, these proteins can be used for the development of highly sensitive and inexpensive diagnostic test systems.

I. M. Sechenov Moscow Medical Academy; *Center for Medical Genetic, Russian Academy of Medical Sciences; **Institute of Transplantology and Artificial Organs, Ministry of Health, Moscow

In the present study changes in protein spectrum and signs of cardiac and renal graft rejection were analyzed using TDE and our previous data [2,6,9].

MATERIALS AND METHODS

The study was performed on autopsy samples of human myocardium (n=100) and kidney (n=15) obtained not later than 3 h after death caused by an incident (control group) and on myocardial (n=4) and kidney (n=6) samples from patients died after allograft transplantation.

Myocardial and renal samples were homogenized in a glass-Teflon homogenizer in 5 volumes of 9.5 M urea, containing 2% Triton X-100, 2% 2-mercaptoethanol, and 2% ampholines, pH 3.5-10 [2,6,9].

Preparation of samples for TDE, TDE fractionation according to O'Farrell with some modifications, and staining with Coomassie blue R-250 and silver nitrate were described previously [9].

0007-4888/98/0002-0162$20.00