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Modification of Biological Activity of Lipopolysaccharide in the Complex with Chitosan

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In the complex with chitosan, lipopolysaccharide partially lost its ability to induce lymphokines tumor necrosis factor and interleukin-8, but retained immunostimulating properties and increased phagocytic function of macrophages by improving digestion of bacteria.

Key Words: *lipopolysaccharide; lipopolysaccharide-chitosan complex; lymphokines; immunity*

Lipopolysaccharides (LPS) are the major component of cell walls in gram-negative bacteria. LPS modulate activity of most systems in infected macroorganisms and induce a variety of pathophysiological changes. Biological effects of LPS are associated with their high toxicity. LPS, endotoxins of gram-negative bacteria, in high doses cause tissue necrosis, severe intoxication, and death of the macroorganism [11]. At the same time LPS are antigens of gram-negative bacteria stimulating cellular and humoral immunity. They modulate functions of macrophages, produce a mitogenic effect on B lymphocytes, activate T lymphocytes, and promote induction of lymphokines by immune cells [4,10]. Much attention is given to the use of LPS in medicine and biotechnology. They serve as adjuvants, inductors of lymphokine synthesis, and antitumor drugs. The search for substances reducing toxicity of endotoxins, but not affecting their immunostimulating activity is an urgent problem.

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We showed for the first time that natural polycation chitosan (CN) form stoichiometrically different complexes with endotoxins. This reaction results in reduction of LPS toxicity [1]. The degree of LPS-induced cell aggregation markedly decreases in the complex with CN.

Here we studied the effect of complex formation on immunobiological activity of LPS. We compared biological properties of LPS from *E. coli* and *Y. pseudotuberculosis* and their complexes with CN.

MATERIALS AND METHODS

LPS were extracted from the microbial mass of *Y. pseudotuberculosis* strain 598 [6]. We also studied LPS from *E. coli* 055:B5 (Sigma). CN (130 kDa) was obtained by alkaline treatment of chitin [1]. The LPS—CN complex (ratio 1:5) was obtained as described elsewhere [1].

The ability of LPS and LPS—CN to induce the synthesis of tumor necrosis factor (TNF) and interleukin-8 (IL-8) was estimated *in vitro* [5]. Venous blood was placed in sterile silicon tubes (14 ml) with 150 U Li-heparin, diluted (1:5) with sterile RPMI-1640 medium containing 300 mg/liter glutamine and 50 μg/ml

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TABLE 1. Effect of *E. coli* LPS, CN, and LPS—CN on the Number of Antibody-Producing Cells in Mouse Spleen (*M*±*m*)

Group	Dose, μg/mouse	Number of antibody-producing cells
Control		38,243.7±2916.5
LPS	50	43,769.3±2910.0*
	20	52,475.9±4171.4*
CN	250	58,502.3±4150.0*
	100	56,614.7±4697.3*
LPS—CN	300	68,330.6±4209.0*
	120	68,327.4±7019.7*

Note. *$p<0.05$ compared to the control.

gentamicin (Gibco Life Technologies GmbH), and incubated with LPS, CN, or LPS—CN at 37°C under gentle shaking. Control incubation was performed with 10 μg/ml LPS from *E. coli*. After 6 h the tubes were placed on ice and centrifuged at 600 rpm for 5 min. Supernatants were collected. Cytokine content was measured using specific test system (DuoSey developing system, Genzyme). We used solutions of LPS (10.0 and 0.5 μg/ml), CN (50.0 and 2.5 μg/ml), and LPS—CN (60 and 3 μg/ml).

Antibody-producing cells in the spleen of (CBA×C57BL)F mice were visualized as described previously [8]. The effects of LPS and LPS—CN on phagocytic activity of mouse peritoneal cells was evaluated as described previously [3]. The animals were intraperitoneally infected with *Y. pseudotuberculosis* strain 512 (10^9 mb/ml). Quantitative and qualitative studies of phagocytosis were performed after 1, 3, and 6 h.

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

RESULTS

The ability of LPS to induce lymphokine synthesis depended on its concentration and structure (Fig. 1, *a*). Independently on the dose, LPS from *E. coli* was more potent than LPS from *Y. pseudotuberculosis*. Low activity of *Y. pseudotuberculosis* LPS is probably related to low acylation of lipid A in this endotoxin [3]. The intensity of monokine synthesis under the influence of *E. coli* LPS was taken as 100%. CN also activated cells and induced the synthesis of IL and TNF. LPS—CN retained the ability to induce *in vitro* production of IL-8 and TNF. However, the ability of *E. coli* LPS in the complex with CN to induce TNF synthesis decreased by more than 2 times (Fig. 1, *a*). The inhibition of TNF synthesis was previously observed during high-specificity binding of LPS to lysozyme [9,12]. However, the complex of *Y. pseudotuberculosis* LPS and CN slightly increased TNF content (compared to LPS, Fig. 1, *a*). These changes were probably related to weak effect of *Y. pseudotuberculosis* LPS. Activity of *Y. pseudotuberculosis* LPS increased in the complex with CN. LPS from *E. coli* and *Y. pseudotuberculosis* partially lost the ability to induce IL-8 production. Activity of LPS from *E. coli* and *Y. pseudotuberculosis* in the complexes with CN decreased by 70 and 20%, respectively (Fig. 1, *b*). These findings are consistent with previous data on the interaction of LPS with other polycations. For example, polymyxin B and lysozyme inhibit IL synthesis after treatment with *E. coli* LPS [7,9]. LPS bound to polycations was less potent in inducing IL synthesis. This was probably associated with modification of physicochemical properties of endotoxin [13].

LPS *in vivo* and *in vitro* stimulates humoral response to antigens. The influence of LPS and LPS—CN complex on the immune response was evaluated by the number of antibody-producing cells in the spleen of mice immunized with sheep erythrocytes. *E. coli* LPS insignificantly increased the number of antibody-producing cells in the spleen (Table 1). However, the count of these cells increased by 2 times after treatment with the LPS—CN complex, which was probably due to high activity of CN. Immunostimulating activity increased under the influence of LPS in both concentrations.

LPS and LPS—CN modulated phagocytic activity of macrophages, which manifested in increased num-

TABLE 2. Effect of *E. coli* LPS, CN, and LPS—CN on Phagocytic Activity of Mouse Peritoneal Cells (M±m)

Sample	Time, h					
	1		3		6	
	PI, %	PN	PI, %	PN	PI, %	PN
Control	49.7±3.2	2.7±0.4	62.6±2.9	4.6±0.7	58.9±5.2	5.2±0.8
LPS	67.0±2.9**	44.3±0.3**	70.0±2.4**	5.4±0.6	42.2±2.3	3.6±0.3**
CN	72.3±4.8*	5.4±1.5	67.4±1.9	4.8±0.3	54.2±2.2	3.0±0.2*
LPS—CN	80.2±3.3*	5.9±0.8**	61.2±2.3	3.4±0.4	36.0±3.4	2.2±0.1*

Note. PI, phagocytic index; PN, phagocytic number. *$p<0.01$ and **$p<0.05$ compared to the control.

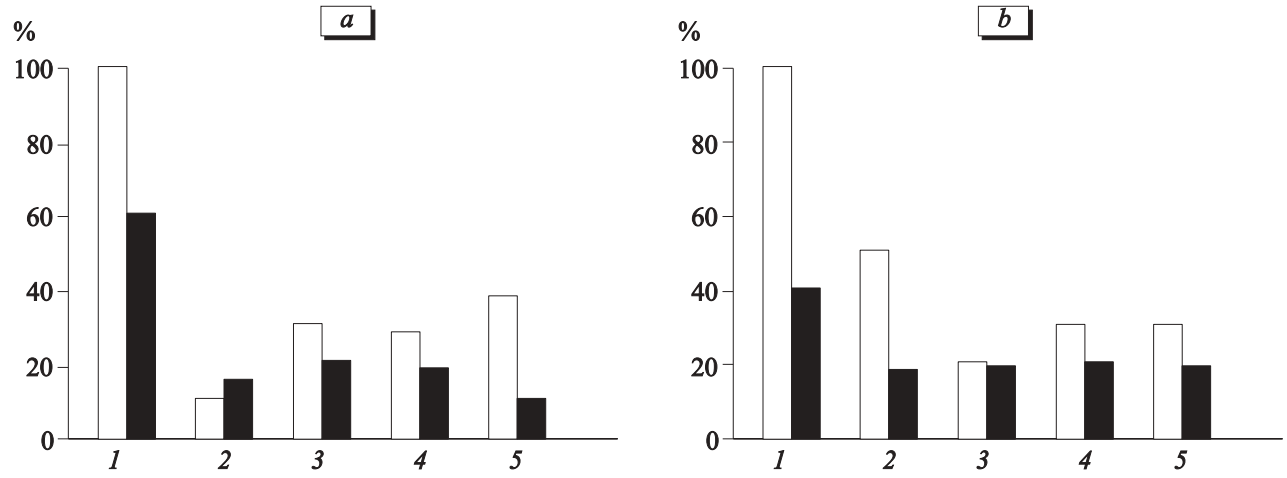

Fig. 1. Contents of TNF (*a*) and IL-8 (*b*) induced by LPS from *E. coli* (*1*) and *Y. pseudotuberculosis* (*2*), chitosan (*3*), and complexes of *E. coli* LPS-chitosan (*4*) and *Y. pseudotuberculosis* LPS-chitosan (*5*). Light bars: 0.5 μg/ml LPS and 2.5 μg/ml chitosan. Dark bars: 10 μg/ml LPS and 50 μg/ml chitosan.

ber (phagocytic number) and activity of phagocytizing cells (phagocytic index). These indexes of phagocytosis increased in control mice by the 3rd and 6th hours, which reflected division of *Y. pseudotuberculosis* (Table 2). However, LPS and LPS—CN decreased phagocytic number and phagocytic index. Therefore, the complex of LPS and CN intensified digestion of microbes. CN bound to LPS did not decrease, but even increased the ability of LPS to improve engulfing activity of macrophages infected with microorganisms.

Our results indicate that CN bound to LPS selectively modulates its biological activity. CN decreases the ability of LPS to induce the synthesis of TNF and IL-8. LPS—CN possesses immunostimulating properties, increases phagocytic function of macrophages, and improves digestion of *Y. pseudotuberculosis*. Modification of biological activity of LPS is probably associated with dissociation of highly aggregated endotoxin particles in the complex with CN [1]. Most authors believe that variations in activity of LPS produced by lysozyme [9,12] and polymyxin [7] result from changes in the macromolecular structure of endotoxins and conformation of active LPS [13]. Changes in biological properties of LPS during its interaction with polycations can be related to shielding of sites responsible for binding to cell receptors. It should be emphasized that CN possesses intrinsic immunobiological activity and can determine biological properties of the complex.

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