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Influence of Lipopolysaccharides and Lipids A from Some Marine Bacteria on Spontaneous and *Escherichia coli* LPS-Induced TNF-α Release from Peripheral Human Blood Cells

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Abstract—Some endotoxic properties of lipopolysaccharides (LPS) and lipids A (LA) from the marine bacteria *Marinomonas communis* ATCC 27118^T, *Marinomonas mediterranea* ATCC 700492^T, and *Chryseobacterium indoltheticum* CIP 103168^T were studied. The preparations tested were shown to have high 50% lethal doses (4 μg per mouse for LPS from *M. mediterranea* and more than 12 μg per mouse for two other LPS and LA from *C. indoltheticum*) and were moderate (371 ± 37 pg/ml at 10 μg/ml of *C. indoltheticum* LPS), weak (148 ± 5 pg/ml at 1 μg/ml of *M. mediterranea* LPS), and zero (LA and LPS from *M. communis* and LA from *C. indoltheticum*) inducers of tumor necrosis factor α (TNF-α) release from peripheral human blood cells. The capacity of the LA and LPS samples from marine bacteria to inhibit TNF-α release induced by LPS from *Escherichia coli* O55 : B5 (10 ng/ml) was also studied.

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Key words: marine proteobacteria, lipopolysaccharide, lipid A, TNF-α

Infectious diseases occupy a significant place (30-35%) in the total mortality of the population in the world. The mortality rate increases to 45% when Gram-negatives participating in development of chronic lung troubles and oncological and cardiovascular diseases act as causative agents [1]. The presence of endotoxins in Gram-negative bacteria cell walls is their distinctive feature. The appearance of endotoxins in the human bloodstream as a result of systemic bacterial infection generates an unregulated activation of the immune system and synthesis of a large quantity of endogenous cytokines by host effector cells [2]. If the level of proinflammatory cytokines such as tumor necrosis factor α (TNF-α) or interleukin-1 remains increased for a long time, "endotoxic shock" progresses [3, 4], presenting a life threat and manifesting in hemorrhagic necrosis, vascular permeability increase, and circulatory collapse, which lead to destruction of tissues and organs [1]. The treatment of "endotoxic shock" still remains a vital issue.

One of the approaches to prevent such conditions is using antagonists of endotoxins, limiting the release or immunological reactivity of the latter [3]. Endotoxins as such are potential candidates for a role of endotoxin antagonists. Chemically, they represent lipopolysaccharides (LPS) including a lipid domain, lipid A (LA) [5], which is considered to be an endotoxic center of LPS [6]. Recent studies have revealed that some nontoxic LA forms block LPS receptors on the target cells and are characterized by strong anti-endotoxic potential [7, 8]. This has stimulated a search for natural sources whose LA have unusual structures and, thereby, lowered endotoxic activity.

LA revealing high antagonism to endotoxins have low acylation and phosphorylation degrees [9] and often occur in bacteria phylogenetically not closely related or distant to the *Enterobacteriaceae* family, the main causative agents of Gram-negative infections. In many respects, the structural peculiarities of LA are formed by

Abbreviations: LA) lipid A; LX) lipid X; LPS) lipopolysaccharide; TNF-α) tumor necrosis factor α.

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bacterial growth conditions. For example, psychrophilic bacteria, preferring low growth temperature, have an under-acylated structural type of LA [10-12]. We supposed that marine bacteria, whose habitat differs from that of terrestrial microorganisms, being characterized by low temperature, high salt concentration, and high hydrostatic pressure, may provide unusual structural forms of LA, and we carried out corresponding investigations [13-16]. As a result, LA from marine Gram-negative bacteria were shown to have specific peculiarities (high homogeneity degree, unique fatty acid composition, mainly pentaacyl and often monophosphoryl types of structure), which distinguish them from LA of terrestrial bacteria. In addition, LA from marine Gram-negative bacteria displayed low toxicity in experiments with animals, which indirectly points to their low endotoxic activity and, theoretically, to their ability to function as endotoxin antagonists.

The present investigation was devoted to elucidation of endotoxic properties of LPS and LA from marine bacteria *Marinomonas communis* ATCC 27118^T, *Chryseobacterium indoltheticum* CIP 103168^T, and *M. mediterranea* ATCC 700492^T, as well as to estimation of the possibility of their use as endotoxin antagonists. These preparations were selected because of the unusual structural peculiarities of LA from marine bacteria—low degree of acylation and phosphorylation, monosaccharide structure type, unusual acylation pattern of glucosaminobiose molecule (Figs. 1a, 1b, and 1c, respectively) are the most pronounced in them.

MATERIALS AND METHODS

Isolation of LPS and LA. The *M. communis* ATCC 27118^T, *M. mediterranea* ATCC 700492^T, and *C. indoltheticum* CIP 103168^T cells were grown at room temperature on rotary shakers in liquid nutrient medium as described earlier [15]. LPS from marine bacteria and *Yersinia pseudotuberculosis* were obtained by the extraction of defatted cells with hot phenol–water mixture according to a published procedure [17]. LPS from *Escherichia coli* O55 : B5 and *Salmonella minnesota* R7 were purchased from Sigma (Germany). LA from *M. communis* and *M. mediterranea* were obtained by hydrolysis of the corresponding LPS (150 mg) with 1% AcOH as described earlier [16]. LA from *C. indoltheticum* was isolated by hydrolysis of defatted bacterial cells (7 g) with 10% AcOH (198 ml, 3 h, 100°C) followed by extraction with $CHCl_3$–MeOH mixture [12]. LA (45 mg) were dissolved in $CHCl_3$–EtOH mixture (3 : 1 v/v) and purified by gel filtration on a Sephadex LH-20 column (500 × 18 mm) using the same mixture.

Induction of TNF-α release. The induction of TNF-α release by the studied preparations was performed *in vitro* in 96-well round-bottomed sterile plastic plates (Greiner, Austria) using human whole blood or mononuclear cells (MNC) from peripheral human blood. Peripheral blood was collected in tubes containing 50 μl of heparin per ml of blood, diluted five times with sterile (cellulose filter Spartan 13/0 0.2 RC, 0.2 μm; Germany) medium containing Hepes (95.3 mg; Sigma), L-glutamine (11.9 mg), benzyl-penicillin (2.5 mg), streptomycin (3.6 mg), gentamicin (100 μl), and 199 medium (36 ml; Sigma), and plated (100 μl aliquots) into each well [18]. MNC were isolated by centrifugation (400*g*, 40 min) of plasma obtained after phase lagging of heparinized blood (50 μl of heparin per ml of blood) for 1 h at 37°C, washed with 199 medium (three times), and resuspended in sterile nutrient medium described above. The resulting cell suspension was plated (50 μl aliquots) into each well containing 20 μl of fetal calf serum. LPS and LA dissolved in sterile water with triethylamine (pH ~ 8) were diluted with medium as needed and plated (100 μl (130 μl in experiments on MNC) aliquots of LPS or LA per well in case of induction of TNF-α release; 50 μl aliquots of LPS and LA per well in case of inhibition of induced TNF-α release). The resulting concentrations of samples in the reaction mixture were 0.1-100,000 ng/ml. The incubation was performed for 22 h at 37°C in 5% CO_2. The comparison of results obtained using MNC and human whole blood did not reveal any significant differences; therefore, the second variant of experiment (as less laborious) was used in this work.

Immunoassays for TNF-α. The content of TNF-α in samples was determined by solid-phase immunoassay using a commercial DuoSet kit system (R&D systems, USA) and 96-well microplate (NUNC, Denmark). The optical density was measured using a μ Quant spectrophotometer (Bio-Tek Instruments, Germany) with wavelength 492 nm.

Statistical analysis. Data were analyzed by Student's *t*-test. The differences between two groups (with and without specimens) were considered to be significant, if $p < 0.05$. The results are presented as M ± SD, where M is the mean of three independent experiments, each of them done in triplicate, and SD is standard deviation.

Lethal toxicity test. Toxic properties of LPS and LA were tested in C-57 line D-galactosamine-sensitized mice (15 g) [19]. The solutions of D-galactosamine-hydrochloric acid (15 mg per animal, in 0.2 ml) and different amounts of LA and LPS (from 0.004 to 40 mg, in 0.2 ml) in phosphate-buffered saline were administrated into groups of four (of three for LPS from *Y. pseudotuberculosis*) animals. Animals in the control group were injected with solution of D-galactosamine in saline (0.2 ml). The death of the mice was confirmed within 48 h. The lethal dose (LD_{50}) was calculated by the Nowotny method [20]: $\log LD_{50} = \log$ (highest dose tested) + $(\log D)[1/2 - \Sigma R/N]$, where ΣR is total number of dead animals, N is number of animals per dose, and D is D-fold difference between successive doses.

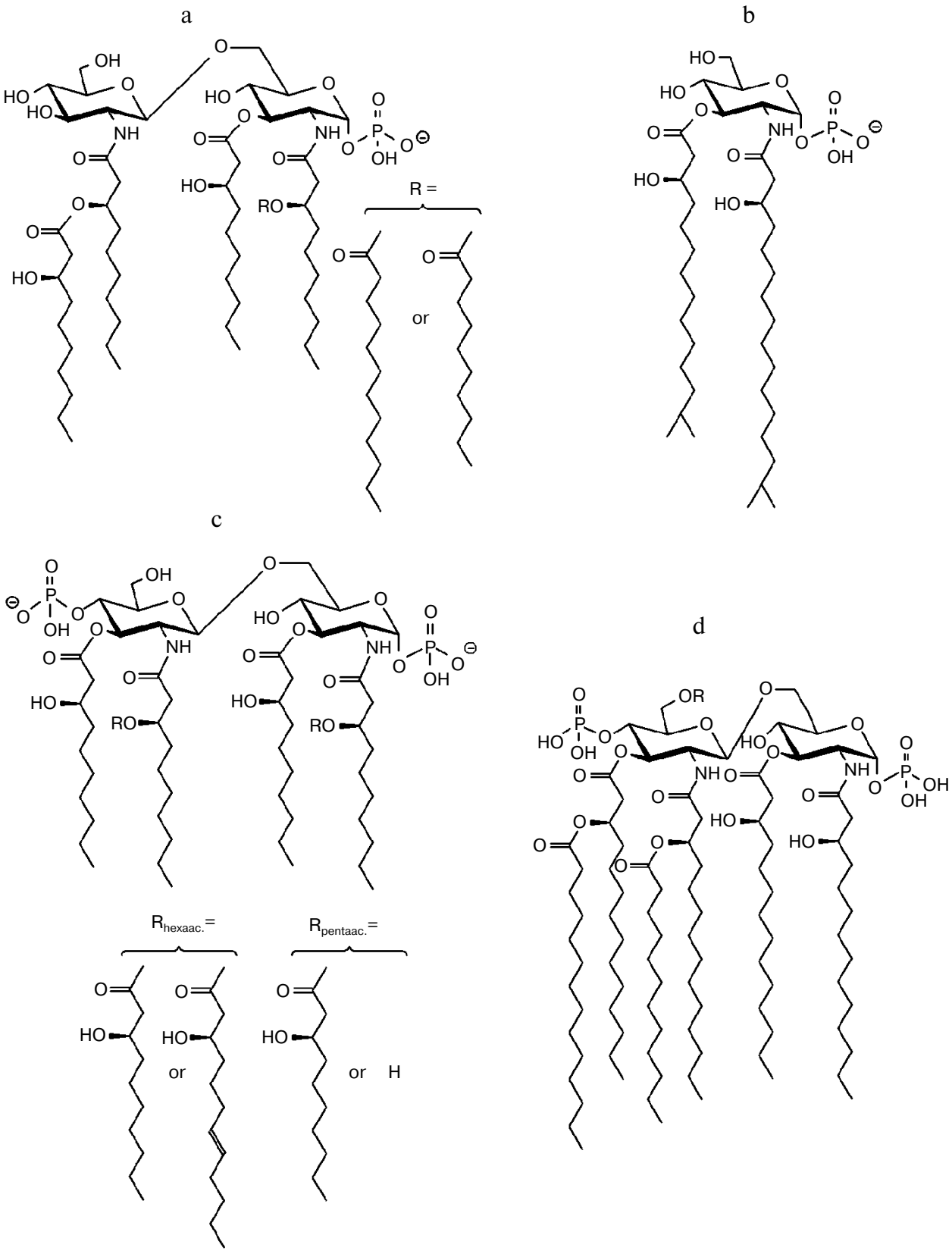

Fig. 1. Structures of LA from *M. communis* ATCC 27118^T [16] (a), *C. indoltheticum* CIP 103168^T (b), *M. mediterranea* ATCC 700492^T (c), and *E. coli* [21] (d).

RESULTS AND DISCUSSION

One of the endotoxic properties of LPS is their toxicity. Therefore at the first stage we determined the acute toxicity of LPS from bacteria *M. communis*, *M. mediterranea*, and *C. indoltheticum* using C-57 line D-galactosamine-sensitized mice [19] (table).

LPS from *M. mediterranea* and especially from *M. communis* displayed low toxicity; their 50% lethal doses were 2.73 and 12.65 μg/mouse, respectively. For comparison, LPS from *Y. pseudotuberculosis* and *E. coli* had greatly lower 50% lethal doses (0.06 and 0.012 μg/mouse, respectively; table). LPS and LA from *C. indoltheticum* were not toxic at all doses studied.

The toxic properties of LPS are associated with their ability to induce TNF-α release from mononuclear human blood cells. Therefore, the ability of the compounds studied to induce and/or inhibit the TNF-α release is often used for characterization of endotoxic and anti-endotoxic potential of various biological preparations [2]. The results of TNF-α release induced by LPS and LA from *M. communis*, *M. mediterranea*, and *C. indoltheticum* in human whole blood are shown in Fig. 2.

LPS from *M. communis* was not active in this test: the content of cytokine in the sample with this compound did not exceed its content in control sample even at the highest concentration of the compound tested (10 μg/ml). LPS from *M. mediterranea* and *C. indoltheticum* started to stimulate TNF-α production at concentration of 100 and 10 ng/ml, respectively (for comparison, the TNF-α release induced by LPS from *E. coli* started at concentration of less then 0.1 ng/ml). Maximum TNF-α production was induced at concentration of 1 and 10 μg/ml of LPS from *M. mediterranea* and *C. indoltheticum*, respectively, but it was 3.2 and 1.4 times lower than that in samples with the same concentrations of LPS from *E. coli*. LA from *M. communis* and *C. indoltheticum* (LA from *M. mediterranea* was not studied in this test) did not induce TNF-α production at all concentrations tested (Fig. 2b).

Thus, LPS and LA from marine Gram-negative bacteria *C. indoltheticum*, *M. communis*, and *M. mediterranea* considerably differ in ability to induce TNF-α production themselves, as from *E. coli* LPS. It is known that endotoxic properties of LPS are determined by structural peculiarities of their lipid domains [6]. LPS, whose LA, like LA from *E. coli* (Fig. 1d) [21], were shown to have two residues of glucosamine, two phosphate groups, and six fatty acid residues, possess maximal endotoxic activity. Any deviation towards increase or decrease of LA acylation degree leads to a significant alteration of its endotoxic activity [9]. As a result, biphosphorylated derivatives of glucosaminobiose with four and five fatty acid residues, respectively, displayed very low activity in stimulation of human immunocompetent cells [22]. It is also known that endotoxic activity of LPS and LA depends both on number of phosphate groups and fatty acid residues [23], and distribution of acyl substituents on the LA molecule [24]. Thus, monophosphorylated LA from *Helicobacter pylori*, *Porphyromonas gingivalis*, and *Flavobacterium meningosepticum* were significantly less active in ability to induce TNF-α release than enterobacterial LPS [25-27]. On the other hand, *Salmonella* spp. and *Pseudomonas aerations* LA, in which the fatty acid residues at positions C3′ and C3, respectively, are missing, had weak endotoxic activities [24, 28].

Among the LA studied, LA from *M. mediterranea* is the most structurally similar to LA from *E. coli* (Fig. 1c). It represents a mixture of two molecular types, which consist of two residues of glucosamine and two residues of phosphoric acid and differ from each other in acylation degree: one part (smaller) of molecules has six fatty acid residues, the other one (the significantly most) has five. In accordance with this, LPS from *M. mediterranea*, which has a small portion of LA molecules with hexaacyl struc-

Acute toxicity of LPS from marine Gram-negative bacteria

Bacterium (preparation)	Number of dead animals (numerator) and total number of animals (denominator) at introduction of the following doses (d) of preparations studied, μg/mouse					LD_{50}, μg/mouse
	d = 0.004	0.04	0.4	4	40	
C. indoltheticum (LPS)	0/4	0/4	0/4	0/4	0/4*	>12
C. indoltheticum (LA)	0/4	0/4	0/4	0/4	n.d.	>12
M. communis (LPS)	0/4	0/4	0/4	0/4	4/4*	12.65
M. mediterranea (LPS)	0/4	0/4	0/4	2/4	n.d.	2.73
Y. pseudotuberculosis (LPS)	0/3	1/3	2/3	3/3	n.d.	0.06
E. coli (LPS)	1/4	3/4	4/4	4/4	n.d.	0.012

Note: n.d., not determined.

* Test samples in 0.2 ml pyrogen-free water were injected intraperitoneally immediately after intraperitoneal administration of 15 mg of D-galactosamine in 0.2 ml pyrogen-free phosphate-buffered saline.

ture type, has considerably lower inducing activity than LPS from *E. coli*.

In contrast to LA from *M. mediterranea*, LA from *M. communis* is a monophosphorylated derivative and does not have acyl substitute at the C3′ atom of the nonreducing end of glucosaminobiose (Fig. 1a). This fact can explain why LA and LPS from *M. communis* did not induce TNF-α production even at maximally high concentrations.

LPS from *C. indoltheticum* appeared to be the most active inducer of TNF-α release among all preparations tested. It should be mentioned that this preparation, which we name "LPS", is apparently not an LPS. Like other LPS tested in this investigation, it was obtained by treatment of *C. indoltheticum* cells with hot aqueous phenol [17]. However, it did not contain 3-hydroxyalkanoic acids, the obligatory components of almost all LPS studied to date [5], and we could not isolate LA from it. The chemical analysis of this so-called LPS showed that it has carbohydrate nature (the content of proteins and nucleic acids in this "LPS" did not exceed 1.5 and 3.2%, respectively) and contains mannose, glucose, glucosamine, galactosamine, 2-aminopentose, and 6-deoxy-3-aminohexose. Thus, the rather high inducing ability of the "LPS" from *C. indoltheticum* is not due to a lipid domain, which is absent from it, but by some other peculiarities of its composition or structure.

Analysis of *C. indoltheticum* fatty acids revealed the presence of 3-hydroxyalkanoic acids in cells of these bacteria, which indirectly points to the existence of LA in them. Using the hydrolysis of bacterial cells with 10% acetic acid [12], we obtained a preparation that contained all the components usually present in LA, namely: glucosamine, 3-hydroxyalkanoic acids, and phosphate groups. Structural study with NMR-spectroscopy and mass-spectrometry of these compound showed that it represents glucosamine monophosphate, acylated with two residues of 3-hydroxyalkanoic acids (Fig. 1b). In contrast to "LPS", LA from *C. indoltheticum* did not induce TNF-α release from human blood cells (Fig. 2b). Structurally it is similar to a biosynthetic precursor of LA, so-called lipid X (LX) [29], and to some synthetic monosaccharide LA analogs known by their low endotoxic activities [30, 31], which is in good agreement with the data obtained.

Thus, the LA and LPS studied appeared to be weak inducers of TNF-α production in human blood cells. It is necessary to note that all endotoxin antagonists based on LA known to date cause practically no induction of cytokine release in mononuclear human blood cells and display weak toxicity [8, 32, 33]. Thus, weakly toxic LA from bacteria *Rhodobacter sphaeroides* suppressed TNF-α release in a dose-depend manner [34], and a synthetic LA analog from *Rhodobacter capsulatus* E-5531 decreased the mortality in experiments with animals [32]. The high 50% lethal doses of LPS and LA from *M. communis* and LA from *C. indoltheticum* in combination with their low inducing ability suggest that theoretically they may be able to function as endotoxin antagonists. The use of endotoxin antagonists, competitive with the former for binding sites of specific receptors on the target cells of the host organism, provides for inhibition of processes leading to development of pathophysiological reactions, in which TNF-α is one of the participants. We studied the ability of these compounds to inhibit the TNF-α release induced by LPS from *E. coli* (at concentration of 10 ng/ml).

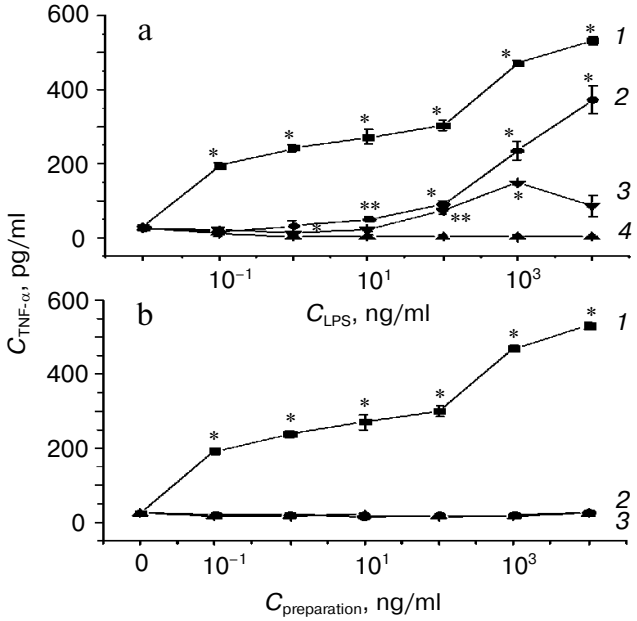

Fig. 2. Induction of TNF-α release in human whole blood samples by LPS (a) and LA (b) from *M. communis* ATCC 27118^T, *M. mediterranea* ATCC 700492^T, and *C. indoltheticum* CIP 103168^T. Heparinized human blood was diluted five times in sterile nutrient medium and plated on plastic 96-well plates (100 μl per well). LPS or LA dissolved in sterile water with triethylamine (pH ~ 8) were plated (100 μl aliquots per well). The samples were incubated for 22 h at 37°C in 5% CO_2. The content of TNF-α in samples was measured by using a commercial immunoassay test system. The results are expressed as M ± SD, where M is the mean of three experiments, each done in triplicate, and SD is standard deviation. The differences between samples with preparations studied and controls were considered significant at * $p < 0.01$, ** $p < 0.02$, and *** $p < 0.05$ (Fig. 3). a: *1-4*) LPS from *E. coli* O55 : B5, *C. indoltheticum*, *M. mediterranea*, and *M. communis*, respectively. b: *1*) LPS from *E. coli*; *2*) LA from *M. communis*; *3*) LA from *C. indoltheticum*.

As evident from Fig. 3, LA from *C. indoltheticum* inhibited TNF-α synthesis by 16.0 and 40.7%, but only at its highest concentrations (10 and 100 μg/ml, respectively). It is interesting that the attempts to find endotoxin antagonists were initially focused on monosaccharide LA analogs. However there are discrepant data concerning their ability to carry out protective functions. Thus, it was shown that LX protected some species of animals from lethal effect of introduced LPS [35, 36], functioned as

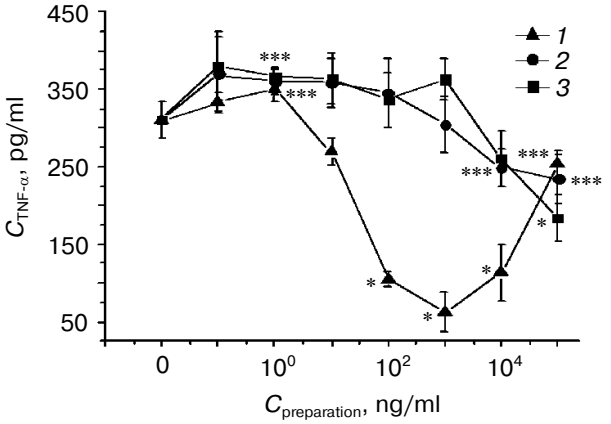

Fig. 3. Inhibition of TNF-α-inducing activity of LPS from *E. coli* O55 : B5 (10 ng/ml) in human whole blood cells by LPS and LA from *M. communis* ATCC 27118^T (*1* and *2*, respectively) and LA from *C. indoltheticum* CIP 103168^T (*3*). Heparinized human blood was diluted five times in sterile nutrient medium and plated on plastic 96-well plates (100 μl per well). LPS and LA dissolved in sterile water with triethylamine (pH ~ 8) were plated (50 μl aliquots per well). Further, the experiment was carried out as described in the legend to Fig. 2.

inhibitor of TNF-α release induced by LPS in some cell systems [37-39], but did not display similar properties in mice and human macrophages, whereas synthetic monosaccharide LA analog completely inhibited TNF-α production induced by 10 ng/ml LPS from *Salmonella abortus equi* at a concentration of 10 μg/ml [30]. It should be mentioned that LX and LA from *C. indoltheticum*, on one hand, and synthetic LA analog, on the other hand, differ from each other in the position of their phosphate groups. Evidently, the differences in biological activity between the synthetic monosaccharide analog and LA from *C. indoltheticum* are explained namely by this.

Despite of the absence of a phosphoric acid residue at the C4′ atom of glucosaminobiose and the presence of only five fatty acid residues, LA from *M. communis* possessed weak inhibitory properties (Fig. 3): at LA concentrations of 10 and 100 μg/ml, the content of TNF-α was reduced by only 19.8 and 24.5%, respectively. In contrast to LA, LPS from *M. communis* displayed pronounced antagonistic properties (Fig. 3). The reduction of TNF-α content under the action of this preparation occurred at a concentration as low as 10 ng/ml. Maximal (almost 80%) inhibition was observed at LPS concentration of 1000 ng/ml. The lack of coincidence in biological activity of the *M. communis* LA and LPS, whose molecules include structurally identical endotoxic centers, can be explained by the fact that LPS, having a hydrophilic O-specific polysaccharide [40], dissolves in water much better then LA (even in the presence of triethylamine).

It should be noted that the increasing concentration of LPS from *M. communis* in a sample led to decrease in its inhibitory activity. As a result, at LPS concentration of 100 μg/ml the content of TNF-α was only by 18% lower than in the control sample. Interestingly, such effect (the decrease in inhibitory activity on increasing the inhibitor concentration) was observed for diphosphorylated *E. coli* LA derivative, having tetraacylated structural type [34]. Perhaps this can be explained by the fact that at high concentration, LPS (and LA) molecules form aggregates (it was shown for instance, that the critical concentrations of aggregate formation for S-form LPS from *S. minnesota*, *S. typhimurium*, and *E. coli* amount to 11, 14, and 22 μg/ml, respectively, and for LA from *S. minnesota* Re595 it is 10 μg/ml [41]). The size of aggregates, their shape, and structure are substantially determined by LPS concentration in solution. It is also established that there is a correlation between aggregate state of endotoxin and its ability to activate (or cancel the activation in case of endotoxin antagonists) immunocompetent cells [42]. However, there is no single opinion concerning the most preferable for displaying agonistic or antagonistic properties aggregate state of LPS and LA. Some authors cite data about higher endotoxic activity of LPS in monomeric form. According to the data of others, aggregates are the biologically active unit of endotoxins during its stimulation of immunocompetent cells [43]. It remains possible that mixed micelles form in reacting medium at high inhibitor concentrations, which leads to significant increase in inducer (LPS from *E. coli*) activity.

It is known that inhibition of inducing activity of endotoxin can be recovered by increasing concentrations of the latter if the inhibition has specific character. To be sure of specificity of inhibitory action of LPS from *M. communis*, we determined whether the inhibition of *E. coli*

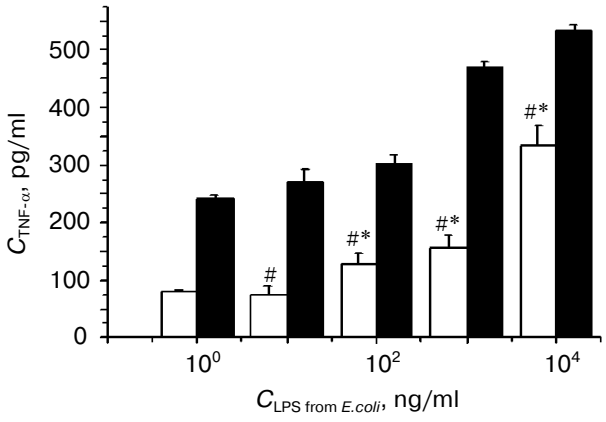

Fig. 4. Increasing concentrations of endotoxin (LPS from *E. coli* O55 : B5) block inhibition by LPS from *M. communis* ATCC 27118^T of induced TNF-α release in human whole blood cells. * $p < 0.05$ when comparing the given sample with previous; # $p < 0.05$ when comparing the given sample with the control sample (with correspondent dose of LPS from *E. coli*). Light columns, LPS from *E. coli* in the presence of 1000 ng/ml of LPS from *M. communis*; dark columns, LPS from *E. coli* without inhibitor. The experiment details are as described in the legend to Fig. 3.

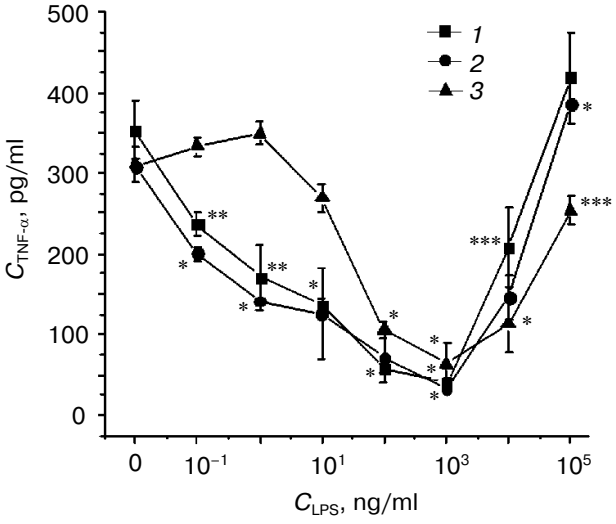

Fig. 5. Inhibition by LPS from *M. communis* of TNF-α release from human whole blood cells induced by LPS from *S. minnesota* R7 (10 ng/ml) (*1*), *Y. pseudotuberculosis* (10 ng/ml) (*2*), and *E. coli* O55 : B5 (10 ng/ml) (*3*). The experiment details are as described in the legend to Fig. 3.

LPS-induced TNF-α release depends on inducer concentration (Fig. 4). It appeared that tenfold increase in the *E. coli* LPS content in the test system led to decrease in inhibition by 15%. When the concentration of inducer reached 10 μg/ml, 40% inhibition was observed. These data indicate that LPS from *M. communis* is apparently a competitive endotoxin inhibitor of LPS from *E. coli*.

LA present in all endotoxins is responsible for proinflammatory cytokine synthesis. It is logical to assume that a potential inhibitor should block effectively the biological activity of any toxic LPS irrespective of its source, length of its O-specific polysaccharide chain, or its toxicity degree. We examined the ability of LPS from *M. communis* to block TNF-α release induced by LPS from *Y. pseudotuberculosis* (LPS of S-form [44], LD_{50} = 0.06 μg/mouse; see table) and *Salmonella minnesota* R7 (LPS of R-form [45]), against the background of already studied LPS from *E. coli* O55 : B5 (LPS of S-form [46], LD_{50} = 0.012 μg/mouse; table). With all endotoxins, LPS from *M. communis* inhibited TNF-α production of human whole blood cells (Fig. 5). These data, as well as the dependence of LPS from *M. communis* inhibitory action on inducer concentration (Fig. 4), are in good agreement with the generally accepted conception that stimulation of immunocompetent cells by endotoxins is carried out via interaction of the latter with receptors specific to LA.

Thus, using human whole blood we showed that LPS and LA from the marine bacteria studied are weak inducers of release of proinflammatory cytokines such as TNF-α. Moreover, our experiments demonstrated that one of the preparations studied, LPS from *M. communis*, is a competitive endotoxin antagonist with a universal type of action. It is interesting that the inhibitory concentrations of LPS from *M. communis* were much lower than those of endotoxin antagonist E-5531, a well known synthetic analog of pentaacylated LA from *R. capsulatus* [33]. For E-5531, semi-maximal inhibition of TNF-α release, induced by 10 ng/ml of LPS from *E. coli*, was observed at concentration of 3765 ng/ml [32], whereas LPS from *M. communis* displayed similar result at concentration lower than 100 ng/ml. This makes further more complex investigation of LPS from *M. communis* as potential endotoxin antagonist promising.

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