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REVIEW

Structural Analysis of Lipopolysaccharides from Gram-Negative Bacteria

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Abstract—This review covers data on composition and structure of lipid A, core, and O-polysaccharide of the known lipopolysaccharides from Gram-negative bacteria. The relationship between the structure and biological activity of lipid A is discussed. The data on roles of core and O-polysaccharide in biological activities of lipopolysaccharides are presented. The structural homology of some oligosaccharide sequences of lipopolysaccharides to gangliosides of human cell membranes is considered.

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Key words: lipopolysaccharide (endotoxin), lipooligosaccharide, lipid A, core, O-antigen, gangliosides, cytokines

Lipopolysaccharides (LPS) from Gram-negative bacteria are amphiphilic molecules composed of a unique structure of lipid A and a large polysaccharide part. Up to $3.5 \cdot 10^6$ LPS molecules can be present on the bacterial cell surface [1]. Among Gram-negative bacteria only *Fibrobacter succinogenes* S85 and diverse members of the genus *Sphingomonas* do not express LPS [2].

Abbreviations: FucNAc, N-acetylfucosamine; GalA, galacturonic acid; GalN, 2-amino-2-deoxygalactose; GalNA, galactosaminuronic acid; GalNAc, N-acetylgalactosamine; GalNAcA, 2-acetamido-2-deoxygalacturonic acid (N-acetyl-D-galactosaminuronic acid); GalNAc3NacA, 2,3-diacetamido-2,3-dideoxygalacturonic acid; GalNAcAN3Ac, 2-acetamido-3-*O*-acetyl-2-deoxygalacturonamide; GalA6(L-Lys), N^{α}-galacturonoyl-L-lysine; GalA6AlaLys, N^{ε}-[(*R*)-1-carboxyethyl]-N^{α}-galacturonoyl-L-lysine; GalNFoAN, 2-formamido-2-deoxygalacturonamide; GlcA, glucuronic acid; GlcNAc, N-acetylglucosamine; Hep7Cm, 7-*O*-carbamoyl-L-*glycero*-D-*manno*-heptose; LBP, LPS-binding mammalian blood glycoprotein; LPS, lipopolysaccharide; mCD14, glycoprotein anchored to membrane surface through glycosylphosphatidylinositol (GPI); MCP-1, monocyte chemotactic protein 1; MD-2, TLR4 associated glycoprotein involved in cell activation by endotoxins; 3-Me-dTal, 3-*O*-methyl-6-deoxytalose; MIP-3α, macrophagal inflammatory protein 3α; MyD88, myeloid differentiation protein 88; Neu5Ac, 5-*N*-acetylneuraminic acid (sialic acid); PGroA, 2-phosphoglyceric acid; QuiNAc, 2-acetamido-2,6-dideoxyglucose; Qui3NAcyl, 3-acylamino-3,6-dideoxyglucose; rhLBP, recombinant human LBP; sCD14, soluble GPI-devoid form of CD14; TLR4, Toll-like transmembrane receptor 4.

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After introduction into mammals LPS exhibit some biological activities, and therefore they are also called **endotoxins**. Lipid A is shown to be a primary factor in inducing the majority of biological effects of LPS [2]. Because of a limited structural variability of the lipid A, all endotoxin molecules for a long time were thought to have a similar, if not identical, biological activity. However, current studies have revealed a number of specific LPS receptors on the surface of immune system cells and also blood proteins such as sCD14 and LBP capable of recognizing the negligible differences in the LPS structure and developing the optimal response of the host [3-11]. Obviously, the biological activity of LPS is determined by its structure. Composition of LPS from different bacterial sources is considered in many works of various authors [12-43]. However, additional biochemical and biophysical studies have revealed new structural features in LPS from free-living marine microorganisms [21, 44] and from bacteria pathogenic for humans and higher plants [2, 29]. Now natural and synthetic lipid A structures with minimal biological activity have been described. They are able to antagonize the effects of toxic LPS. This review covers data on relationships between the structure and biological activity of endotoxins.

Lipid A is a structural component of LPS that is responsible for its biological activity [45]. Lipid A structure is subdivided formally into a *hydrophilic backbone* consisting of carbohydrate components with substituents (so-called head-groups) and a *hydrophobic moiety* consisting of fatty acid residues. The lipid A from *Escherichia*

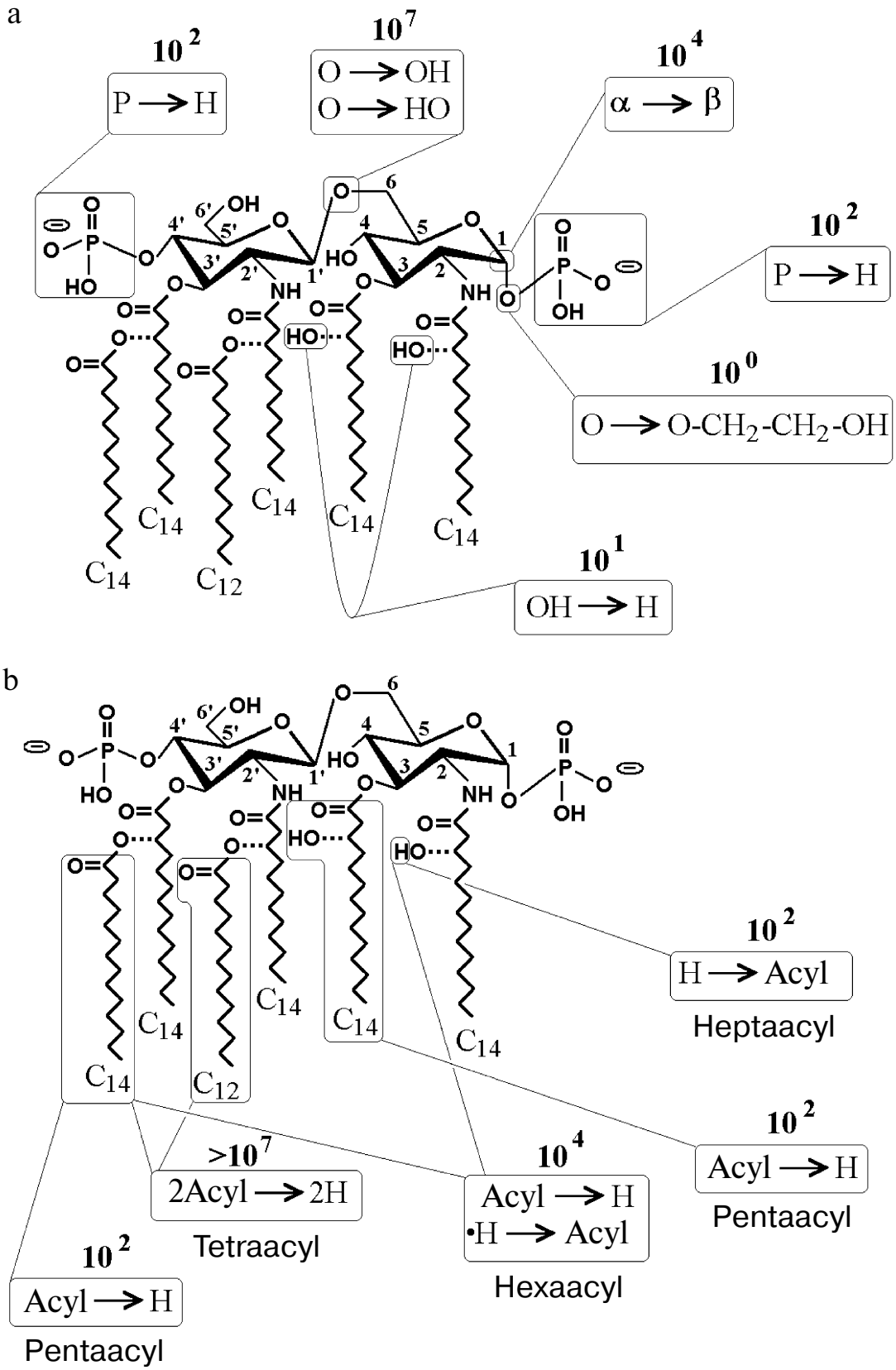

Fig. 1. Effects of structural modifications of *E. coli* lipid A on its biological activity [46]. The factor of decrease in biological activity is presented as 10^n.

coli LPS is a classical example. Its backbone consists of β(1-6)-linked D-glucosamine units (GlcNI-GlcNII) carrying phosphate groups at O-4′ position of GlcNII and at O-1 position of GlcNI (Fig. 1). In addition, four (R)-3-hydroxytetradecanoyl residues are directly linked to positions 3 and 3′ and to the amino groups of both glucosamines. (R)-3-hydroxyacyl chains at O-3′ and N-2′ positions of GlcNII are etherified with tetradecanoic and dodecanoic acid, respectively. The hydroxyl group at O-4 position of he reducing glucosamine is not substituted [46] (Fig. 1).

The carbohydrate backbones of lipids A characterized to date contain D-*gluco*-configurated hexosamine residues: 2-amino-2-deoxy-D-glucopyranose (D-Glc*p*N or glucosamine [GlcN]) or 2,3-diamino-2,3-dideoxy-D-glucopyranose (D-Glc*p*N3N, GlcN3N, or DAG), which are present as β(1-6)-linked homodimers (GlcNII-GlcNI; GlcN3NII-GlcN3NI) or a heterodimer (GlcN3NII-GlcNI) [2, 47]. A disaccharide backbone of the GlcN3NII-GlcNI type is found in the lipid A from the marine bacterium *Arenibacter certesii* KMM 3941^T [48]. The lipid A backbone from *Rhizobium leguminosarum* and *Rhizobium etli* has an unusual structure: instead of GlcNI it includes 2-amino-2-deoxygluconic acid residue [27, 49]. Natural monosaccharide backbone structures of the GlcN3N type are found in lipids A from *Blastochloris* (*Rhodopseudomonas*) *viridis*, *Blastochloris* (*Rhodopseudomonas*) *sulfoviridis*, and *Rhodopseudomonas palustris* [50] while those of the GlcN type – in lipids A from *Chryseobacterium* (*Flavobacterium*) *indoltheticum* [44] and *Chryseobacterium scophtalmum* CIP 104199^T [48, 51].

Lipid A is linked to 3-deoxy-D-*manno*-octulosonic acid residue (ketodeoxyoctonic acid (KDO)) through a hydroxyl group at the O-6′ position of GlcNII and as a result to core region of LPS [47]. Lipids A from different bacteria have the same structure but differ in the head-group substituents and in the number and composition of fatty acids. Table 1 presents the most widespread substituents at the O-1 and O-4′ in lipid A disaccharide. As follows from the data presented in Table 1, bisphosphory-

Table 1. Substituents at the O-1 and O-4′ positions of the lipid A backbone

Bacterium	Substituent		References
	O-4' GlcN II	O-1 GlcN I	
Actinobacillus actinomycetemcomitans PO 1021-7	P	P	[52]
A. addita KMM 3600^T	P	P	[53]
Bordetella bronchiseptica, B. hinzii, B. parapertussis, B. pertussis	P	P	[54]
B. multivorans	P/Ara*p*4NP	Ara*p*4NP	[55]
B. cenocepacia	P	P/Ara*p*4NP	[56]
C. testosteroni	P	P	[25]
C. trachomatis	P	P	[57]
E. carotovora FERM P-7576	P	P	[29]
F. tularensis 1547-57	H	GalNP	[58]
F. nucleatum JCM 8532 (ATCC 25586)	P	P	[8]
H. pylori 26695	H	$PEtNH_2$	[59]
K. pneumoniae R20	P	P	[60]
N. meningitidis M986-NCV1	$PPEtNH_2$	$PPEtNH_2$	[61]
P. gingivalis SU63	P/H	P/$PEtNH_2$	[62]
P. mirabilis R45	P/Ara*p*4NP	P	[63]
P. aeruginosa	H/P/Ara*p*4NP	P	[47, 64, 65]
R. fulvum DSM117	Hep	GalA	[66]
S. typhimurium	Ara*p*4NP	$PPEtNH_2$	[67]
S. marcescens AU01	P	P	[68]
	O-4' GlcN3N II	O-1 GlcN3N I	
B. henselae ATCC 49882^T	P	P	[5]
Mesorhizobium huakuii IFO 15243^T	P/H	GalA	[20]

Note: PP, pyrophosphate; $PEtNH_2$, 2-aminoethylphosphate; $PPEtNH_2$, 2-aminoethylpyrophosphate; P-CH_3, methylphosphate; GalA, D-galacturonic acid; Ara*p*NP, 4-amino-4-deoxy-L-arabinosophosphate; Hep, heptose; GalNP, galactosamine-1-phosphate.

lated lipids A have significant negative charge. Phosphate groups are fully substituted by negatively charged D-galacturonic acid residues in the lipid A from the hyperthermophilic bacterium *Aquifex pyrophilus* [28]. Negative charge of the lipid A from *R. etli* and *R. leguminosarum*, which does not have phosphate groups, is due to galacturonic acid residue at O-4′ position and/or GlcNI oxidized to 2-aminogluconic acid [27, 69]. Substituents at the O-1 and O-4′ positions of the lipid A disaccharide backbone determine the total charge of the molecule.

The number of fatty acids within natural lipids A varies from four in those from *Francisella tularensis*, *Helicobacter pylori*, and *Pseudoalteromonas issachenkonii* KMM 3549^T, which produce tetraacyl lipids A, to seven in heptaacyl lipids A from *Erwinia carotovora* [29, 48, 70] and *Acinetobacter radioresistens* S13 [71]. In most hexaacyl lipids A, fatty acid residues are distributed asymmetrically between glucosamines of the lipid A (Table 2). Fatty acids are distributed symmetrically [2+2] in the lipid A from *Coxiella burnetii* and some hexaacylated

Table 2. Distribution of fatty acid residues between hydroxyl and amino groups of the lipid A backbone

Bacterium	GlcN II		GlcN I		References
	O-3'	N-2'	O-3	N-2	
A. actinomycetemcomitans PO 1021-7	14:0[3-O(14:0)]	14:0[3-O(14:0)]	14:0(3-OH)	14:0(3-OH)	[52]
A. addita KMM 3600^T	12:0(3-OH)	14:0[3-O(12:0)]	10:0(3-OH)	14:0(3-OH)	[53]
B. bronchiseptica	14:0[3-O(16:0)]	14:0[3-O(14:0)]	12:0(3-OH)	14:0(3-OH)	[54]
B. hinzii	14:0[3-O(16:0)]	14:0[3-O(14:0 (2-OH))]	12:0(3-OH)	14:0(3-OH)	
B. parapertussis	10:0(3-OH)	14:0[3-O(14:0)]	16:0	14:0(3-OH)	
B. pertussis	14:0(3-OH)	14:0[3-O(14:0)]	10:0(3-OH)	14:0(3-OH)	
B. multivorans, B. cenocepacia	14:0(3-OH)	16:0[3-O(14:0)]	14:0(3-OH)	16:0(3-OH)	[55, 56]
C. trachomatis	14:0–16:0	20:0[3-O (18:0–21:0)]	14:0/15:0	20:0(3-OH)	[57]
E. carotovora FERM P-7576	14:0[3-O(12:0)]	14:0[3-O(12:0)]	14:0(3-OH)	14:0[3-O(16:0)]	[29]
F. tularensis 1547-57	H	18:0[3-O(16:0)]	18:0(3-OH)	18:0(3-OH)	[58]
F. nucleatum JCM 8532 (ATCC 25586)	14:0[3-O(14:0)] 16:0[3-O(14:0)]	16:0[3-O(14:0)]	14:0(3-OH)	16:0(3-OH) 14:0(3-OH)	[8]
H. pylori 26695	H	18:0[3-O(18:0)]	16:0(3-OH)	18:0(3-OH)	[59]
K. pneumoniae R20	14:0[3-O(14:0)]	14:0[3-O(14:0)]	14:0(3-OH)	14:0[3-O(16:0)]	[60]
P. gingivalis	i15:0(3-OH)	i17:0[3-O(16:0)]	16:0(3-OH)	i17:0(3-OH)	[62]
P. mirabilis R45	14:0[3-O(14:0)]	14:0[3-O(14:0)]	14:0(3-OH)	14:0(3-OH)	[63]
P. aeruginosa	10:0(3-OH)	12:0[3-O(12:0 (2-OH))]	H	12:0[3-O(12:0 (2-OH))]	[47]
R. fulvum DSM117	14:0[3-O(12:0)]	14:0[3-O(16:0)]	14:0(3-OH)	14:0(3-OH)	[66]
S. enterica sv. Minnesota R595	14:0[3-O(14:0)]	14:0[3-O(12:0)]	14:0(3-OH)	14:0[3-O(16:0)]	[72]
S. minnesota	14:0[3-O(14:0)] 14:0[3-O(14:0 (2-OH))]	14:0[3-O(12:0)]	14:0(3-OH) 14:0[3-O(16:0)]	14:0(3-OH)	[47]
S. typhimurium	14:0[3-O(14:0)]	14:0[3-O(12:0)]	14:0(3-OH)	14:0(3-OH)	[47]
S. marcescens AU01	14:0[3-O(14:0)]	14:0[3-O(14:0)]	H	14:0(3-OH)	[68]

Table 3. Composition of fatty acid residues in hexaacyl [3+3] lipids A

Bacterium	GlcN II			GlcN I			References
	O-4'	O-3'	N-2'	O-3	N-2	O-1	
C. burnetii strain Priscilla	P	16:0 15:0	16:0(3-OH) i16:0(3-OH)	16:0	16:0(3-OH) i16:0(3-OH)	P	[24]
C. testosteroni	P	10:0(3-OH)	10:0[3-O (12:0)]	10:0(3-OH)	10:0[3-O (14:0)]	P	[25]
Sh. sonnei	P	14:0(3-OH)	14:0[3-O (14:0)]	14:0(3-OH)	14:0[3-O (12:0)]	P	[73]
R. gelatinosus Dr_2	P	10:0(3-OH)	10:0[3-O (12:0)]	10:0(3-OH)	10:0[3-O (12:0)] 10:0[3-O (14:0)]	P	[74]
C. violaceum	P	10:0(3-OH)	12:0[3-O (12:0)]	10:0(3-OH)	12:0[3-O (12:0)]	P	[47]
N. meningitidis M986-NCV1	$PPEtNH_2$	12:0(3-OH)	14:0[3-O (12:0)]	12:0(3-OH)	14:0[3-O (12:0)]	$PPEtNH_2$	[61]
	GlcN3N II			GlcN3N I			
A. pyrophilus	GalA	14:0[3-O (18:0)]	16:0(3-OH)	14:0(3-OH)	16:0(3-OH)	GalA	[28]
B. abortus	H/P	16:0[3-O (28:0 (27-OH))]	14:0(3-OH)	14:0[3-O (18:0)]	12:0(3-OH)	H/P	[75]
L. interrogans	H	12:0(3-OR)	16:0(3-OR)	12:0(3-OH)	16:0(3-OH)	PCH_3	[23]

Note: R, cis$\Delta^5$12:1 or cis$\Delta^7$14:1.

[3+3] structures (Table 3). Endotoxins from the marine bacteria *Pseudoalteromonas haloplanktis* TAC 125 and *Alteromonas addita* KMM 3600^T contain pentaacyl lipids A (Table 2) [48, 53, 76].

Most often the hydrophobic moiety of lipids A is formed by fatty acids containing from 10 (*Rubrivivax* (*Rhodocyclus*) *gelatinosus*, *Comamonas testosteroni*) to 14 carbon atoms (*Rhodospirillum fulvum*, *Proteus mirabilis*, *Salmonella enterica*, *E. carotovora*, etc.). Data presented in Tables 2 and 3 indicate that the most frequent component of lipids A is 3-hydroxytetradecanoic acid. Dodecanoic, tetradecanoic, and hexadecanoic acids are other fatty acids found in different lipids A. These three fatty acids, together with 3-hydroxytetradecanoic acid, are components of the recently characterized lipid A of endotoxin from *Rahnella aquatilis* 2-95 [77]. Other lipids A contain 3-hydroxynonanoic or 3-hydroxydodecanoic acids. The hexaacylated structure of the lipid A from *Shewanella pacifica* KMM 3772 is formed by 3-hydroxytridecanoic and tridecanoic acids only (Table 4). In the lipids A from *F. tularensis*, *H. pylori*, and *Chlamydia trachomatis* hydrocarbon chains of the fatty acids include 18-20 carbon atoms (Table 2). A rare 27-hydroxymontanoic acid has been found in the lipids A from *R. etli* and *Rhizobium* sp. Sin-1 (Table 4). In the lipid A from *R. etli* CE3 the hydroxyl group at C-27 forms ester bond with 3-hydroxybutyric acid. This functional group has been also identified in the lipid A from *Sinorhizobium meliloti* [82].

The lipids A from *Porphyromonas* (*Bacteroides*) *gingivalis* (Table 2), *Chryseobacterium* (*Flavobacterium*) *meningosepticum* [84], and *Bacteroides fragilis* (Table 4) are characterized by the presence of 13-methyltetradecanoic, 3-hydroxy-13-methyltetradecanoic, and 3-hydroxy-15-methylhexadecanoic iso-acids. 2-Hydroxyalcanoic acids are mainly found as second acylating components in acyloxyacyl groups of lipids A from *Pseudomonas reactans* [22], *Pseudomonas aeruginosa*, and *Bordetella hinzii* (Table 2). 2,3-Dihydroxyalcanoic and 2,3-dihydroxyisoalcanoic acids are constituents of the lipid A of LPS from *Legionella pneumophila* (Table 4).

A rare 3-oxotetradecanoic acid has been detected only in the lipids A from the phototrophic bacteria *Rhodobacter* (*Rhodopseudomonas*) *capsulatus* and *Rhodobacter* (*Rhodopseudomonas*) *sphaeroides* [79, 80, 85]. Lipids A of some bacteria include unsaturated acids; thus, the lipid A from *Rb. sphaeroides* contains unsaturat-

Table 4. Unusual fatty acids in the composition of lipids A

Bacterium	GlcN II			GlcN I			References
	O-4'	O-3'	N-2'	O-3	N-2	O-1	
B. fragilis NCTC 9343	H	16:0(3-OH)	i17:0[3-O (i15:0)]	15:0(3-OH)	16:0(3-OH)	P	[78]
M. vaga ATCC 27119^T	H	H	10:0[3-O (10:0(3-OH))]	10:0(3-OH)	10:0[3-O (10:0)] 10:0[3-O (12:0)] 10:0[3-O (cis$\Delta^5$12:1)]	P	[21]
R. etli CE3	GalA	14:0(3-OH)	14:0[3-O (27-O(4:0(3-OH))28:0)]	14:0(3-OH) H	14:0(3-OH)	H	[27, 69]
Rb. capsulatus 37b4	$PEtNH_2$	10:0[3-O (cis$\Delta^5$12:1)]	14:0(3-oxo)	10:0(3-OH)	14:0(3-oxo)	$PPEtNH_2$	[79]
Rb. sphaeroides	P	10:0(3-OH)	14:0[3-O (cis$\Delta^7$14:1)] 14:0[3-O (14:0)]	10:0(3-OH)	14:0(3-oxo)	P	[80]
S. pacifica KMM 3772	P	13:0[3-O (13:0)]	13:0[3-O (13:0)]	13:0(3-OH)	13:0(3-OH)	P	[81]
S. meliloti	P	14:0(3-OH)	16:0[3-O (29-O(4:0(3-OH))30:0)]	14:0(3-OH)	16:0(3-OH)	P	[82]
	GlcN3N II			GlcN3N I			
B. henselae ATCC 49882^T type 1	P	12:0(3-OH)	16:0[3-O (26:0(25-OH))] 16:0[3-O (28:0(27-OH))]	12:0(3-OH)	16:0(3-OH)	P	[5]
B. henselae ATCC 49882^T type 2	P	12:0[3-O (26:0(25-OH))] 12:0[3-O (28:0(27-OH))]	16:0(3-OH)	12:0(3-OH)	16:0(3-OH)	P	
L. pneumophila strain Philadelphia 1	P	i14:0[3-O (28:0(27-oxo))] i14:0[2-OH, 3-O(28:0(27-oxo))]	18:0[3-O (i16:0)] 20:0[3-O (i16:0)] 22:0[3-O (i16:0)]	i14:0(3-OH) i14:0(2,3-di-OH)	18:0(3-OH) 20:0(3-OH) 22:0(3-OH)	P	[83]
Mesorhizobium huakuii IFO 15243^T	H/P	i13:0[3-O (20:0)] i13:0(3-OH)	20:0[3-O (28:0(27-OH))] 20:0(3-OH)	i13:0 (3-OH)	20:0(3-OH)	GalA	[20]

Table 5. Composition and distribution of fatty acid residues and phosphate groups in synthetic analogs of natural lipids A

Synthetic analog	GlcN II			GlcN I			References
	O-3'	N-2'	O-4'	O-3	N-2	O-1	
Compound 406 (LA-14-PP, Ia, IVa)	14:0(3-OH)	14:0(3-OH)	P	14:0(3-OH)	14:0(3-OH)	P	[90]
Compound 503	14:0[3-O(14:0)]	14:0[3-O(12:0)]	H	14:0(3-OH)	14:0(3-OH)	H	[91]
Compound 504 – analog of lipid A from *E. coli*	14:0[3-O(14:0)]	14:0[3-O(12:0)]	P	14:0(3-OH)	14:0(3-OH)	H	[91]
Compound 505 – analog of lipid A from *E. coli*	14:0[3-O(14:0)]	14:0[3-O(12:0)]	H	14:0(3-OH)	14:0(3-OH)	P	[91]
Compound 506 (LA-15-PP) – analog of lipid A from *E. coli*	14:0[3-O(14:0)]	14:0[3-O(12:0)]	P	14:0(3-OH)	14:0(3-OH)	P	[90, 91]
Compound 516 (LA-16-PP) – analog of lipid A from *S. minnesota*	14:0[3-O(14:0)]	14:0[3-O(12:0)]	P	14:0(3-OH)	14:0[3-O(16:0)]	P	[90, 91]
Compound LA-21-PP	14:0(3-OH)	14:0[3-O(16:0)]	P	14:0(3-OH)	14:0(3-OH)	P	[92]

ed Δ^7-tetradecanoic acid, and Δ^5-dodecanoic acid has been identified in lipids A from *Rb. capsulatus* and *Marinomonas* (*Alteromonas*) *vaga* (Table 4). 3-Hydroxy fatty acids at positions O-3′ and N-2′ in the lipid A from *Leptospira interrogans* are acylated with Δ^5-dodecanoic or Δ^7-tetradecanoic acids (Table 3). Oleic, palmitoleic, and linolenic acids are components of the lipid moiety of LPS-like molecules isolated from cyanobacteria [86]. The lipid moiety of LPS from the cyanobacteria *Anacystis nidulans* may include dodecanoic, 2-hydroxytetradecanoic, hexadecanoic, docosanoinic (C22:0), and linoleic (C18:2) fatty acids [87].

Unique long chain fatty acids with hydroxyl group at the ω-1 position of the hydrocarbon chain are present in the lipids A from almost all bacteria of the Rhizobiaceae family [88, 89] and also in the lipids A from intracellular pathogens of mammals – *Brucella abortus* and *Bartonella henselae* (Tables 3 and 4). Fatty acids C28:0 (27-keto) and C28:0 (27-hydroxy) are found in the lipid A from *L. pneumophila* [83].

Several blood proteins, such as the LPS-binding protein (LBP) and a soluble form of LPS receptor (sCD14), are supposed to recognize the lipid A structure upon its interaction with endotoxins [8, 93-95]. In particular, the synthetic compound 506, which is a structural analog of the lipid A from *E. coli* with a small difference in the fatty acid composition, displayed higher lethal toxicity in mice than the lipid A of LPS from *Fusobacterium nucleatum* sp. *nucleatum* [8]. In the absence of serum, both lipids A were similar in the *in vitro* activation of the nuclear factor NF-κB in human cells, but their activities were significantly different in the presence of plasma, CD14, or sCD14 and LBP. The authors think this difference is caused by the weaker interaction of the lipid A from *F. nucleatum* with sCD14, and data of native electrophoresis/Western immunoblotting has confirmed their explanation [8]. Differences in the length of fatty acids of the lipid A from *F. nucleatum* and compound 506 are presented in Tables 2 and 5. LPS from *L. pneumophila*, whose lipid A consists of isoalcanoic and unique fatty acids (C20, C22, C28), does not interact with sCD14 [96] (Table 4). It is likely that an increase in the length of fatty residues exceeding their length in lipids A from enterobacteria may disturb the interaction of sCD14 with the hydrophobic component of the lipid A. This is biologically important because LBP catalyzes LPS delivery to mCD14 and promotes endotoxin binding with sCD14 [97, 98], and the interaction of sCD14 with LPS increases susceptibility to endotoxins of cells not expressing mCD14 [97]. Similar data on the influence of the lipid A composition on its interaction with LBP were obtained in the work [94]. LPS from *E. coli* BMS A016 bound with rhLBP at endotoxin concentration lower than 10 ng/ml, whereas a pronounced interaction of other LPS with LBP could be observed at higher concentrations – more than 500 ng/ml for LPS from *H. pylori* and 1000 ng/ml for LPS from *P. gingivalis*. Moreover, the binding of LPS from *H. pylori* and *P. gingivalis* with LBP occurred significantly more slowly than the binding of LPS from *E. coli* BMS A016 [94]. The binding affinity of the tetraacylated Re-structure of the endotoxin from *Chlamydophila psittaci* 6BC containing C20- and C21-fatty acids with rhLBP was at least fivefold

lower than the binding affinity of LPS from *E. coli* O111:B4 [99, 100]. LPS from *F. tularensis* LVS ATCC 29684 containing tetraacyl lipid A (C16, C18) (Tables 1 and 2) also has virtually no interaction with LBP [7, 12, 58]. In addition to the presence of fatty acids with hydrocarbon chain longer than 14 carbon atoms, the charge of lipids A from *H. pylori*, *P. gingivalis*, *F. tularensis* was lower than the charge of lipids A from *E. coli* and compound 506 [58, 59, 62], which could also affect the binding. The low affinity of LPS binding with LBP and/or sCD14 is likely to influence the rate of endotoxin delivery to membranes of target cells and as a result to decrease the effectiveness of LPS signalization [7, 100].

There is now no doubt that lipid A is a biologically active component of endotoxin [90, 91, 101, 102]. To elucidate the contribution of lipid A constituents to its biological activity, a number of lipid A analogs lacking different functional groups have been synthesized (Table 5). Figure 1a shows the influence of modification of substituents in the disaccharide backbone of lipid A on the biological activity of the molecule [1]. The monosaccharide form of lipid A is even less active (its activity is decreased more than 10^7 times) [103]. Comparison of abilities of the compounds 504, 505, and 506 to induce IL-1 synthesis by human monocytes revealed the role of lipid A charge in its biological activity [90]. Modifications in the hydrophobic region of the molecule (Fig. 1b) also strongly influence lipid A biological activity. Removal of a fatty acid resulting in pentaacyl lipid A or introduction of an additional fatty acid residue (heptaacyl lipid A) decreased the biological activity 10^2-fold. A tetraacyleted precursor of lipid A known as compound 406 failed to induce cytokine production by human monocytes. The biological activity of lipid A is associated not only with the number of fatty acids, but also with their length. Thus, the lipid A of LPS from *H. pylori* contains 3-hydroxyhexadecanoic and 3-hydroxyoctadecanoic fatty acids, and its ability to activate THP-1 cells for synthesis of TNF-α is lower than that of LPS from *E. coli* O55:B5 [83]. In experiments on Mono Mac 6 cell line, the minimal activity was displayed by the hexaacyl lipid A of LPS from *L. pneumophila* containing 27-oxomontanoic, 3-hydroxyisotetradecanoic, and isohexadecanoic acids [96]. The lipid A from *Rhizobium* Sin-1 containing a unique acyloxyacyl residue 16:0[3-O(28:0(27-OH))] and 2-aminogluconic acid instead of GlcNI did not stimulate TNF-α production by human monocytes [104]. In experiments with human whole blood, LPS from *B. henselae* whose pentaacyl lipid A contains an acyloxyacyl residue 16:0[3-O(28:0(27-OH))] also did not induce the release of TNF-α [105].

Recent works have shown that the spectrum of cytokines released by blood cells in response to endotoxins is rather diverse and depends on the LPS composition (Table 6). Thus, LPS from *Brevundimonas* (*Pseudomonas*) *diminuta* induces synthesis of IL-1 and IL-6 but does not stimulate production of TNF-α by human mononuclear cells [112]. Differences in the spectra of released cytokines can be explained by both primary lipid A structure of the endotoxin and the origin of cells used in experiments. For example, the lipid A from the *Salmonella* genus activated mouse macrophages but not human cells, whereas the lipid A from *E. coli* equally activated mouse and human cells [9]. The same was observed for compound 406, which behaved as an agonist for mouse and rabbit cells but could not activate human cells [90, 113]. The lipid A from *L. interrogans* did not activate human THP-1 cells but stimulated the production of TNF-α by RAW 264.7 mouse cells [23]. These data represent the species-specific effect of endotoxins and suggest a difference in both recognizing mechanisms by cells and their responses to different lipid A structures [54]. Biological activities of lipids A of endotoxins have been studied using different cell types and different media (Tables 2-4); therefore, their absolute assessment is difficult. Analysis of data presented in the works of Tanamoto et al. has

Table 6. Influence of LPS structure on cytokine production by human phagocytes [54]

Source of LPS	Cytokine	Cell type	References
B. pertussis	IL-1	monocytes	[106]
V. cholerae	IL-1	monocytes	[107]
N. meningitidis	IL-1	monocytes	[108]
Actinobacillus actinomycetemcomitans	IL-1, TNF-α, IL-6, IL-8	monocytes	[109]
P. gingivalis	IL-1, TNF-α, IL-6, IL-8	monocytes	[109]
P. aeruginosa	TNF-α	cell line Mono Mac 6	[110]
L. pneumophila	IL-1β, TNF-α, IL-6, IL-8	cell line Mono Mac 6	[96]
Burkholderia cepacia	TNF-α	cell line Mono Mac 6	[110]
H. pylori	IL-1, TNF-α	mononuclear cells	[111]

shown that abilities of lipids A (100 ng/ml) with different structure to activate differentiated THP-1 cells for TNF-α synthesis is decreased in the following sequence: compound 506 > *C. meningosepticum* > *C. testosteroni* > *Salmonella minnesota*, *Salmonella typhimurium*, *Salmonella abortus equi*, compound 516 [9, 25, 114].

Endotoxins extracted from bacterial strain cultivated *in vitro* and those extracted from the same strain immediately upon its isolation from conditions *in vivo* can differ in their composition [115]. Thus, the lipid A from *Burkholderia* (*Pseudomonas*) *multivorans* isolated from the *in vitro* bacterial culture is represented by proportional mixture of penta- and tetraacylated structures containing two 4-amino-4-deoxy-L-arabinose residues. The mixture of endotoxins from *B. multivorans* isolated from the strain upon its being transferred under *in vivo* conditions contained a significant amount of LPS with tetraacyl lipid A and with only one 4-amino-4-deoxy-L-arabinose residue. Modifications in the structure of endotoxin from *B. multivorans* influenced its biological activity. The more heterogenous mixture of the endotoxin structures more actively induced TNF-α release from the human monoblastic cell line U937 than endotoxins isolated from this strain cultivated *in vivo* [55].

Alteration of biological activity was shown for endotoxins from *Yersinia pestis* cultivated at 27 or 37°C [116]. The activity of LPS from *Y. pestis* in induction of TNF-α production by differentiated U937 cells was higher for LPS isolated from the cells grown at the lower temperature. Differences in the biological activity correlated with the lipid A structure. At physiological temperature *Y. pestis* produced the bisphosphorylated tetraacyl lipid A consisting of 3-hydroxytetradecanoic acid residues only [116, 117]. The hexaacyl lipid A from *Y. pestis* LPS obtained from cells grown at 27°C was supplemented by acyloxyacyl residues 14:0[3-O(12:0)] and 14:0[3-O(16:1)] and by substitution of the phosphate residue at the O-4′ position of GlcNII by 4-amino-4-deoxy-L-arabinosophosphate also [116]. Changes in the growth temperature also influence the core region structure of endotoxins from *Y. pestis*. The *Y. pestis* strains cultivated at 37°C produced only one glycoform of the core. At 25°C four glycoforms of the core which were different in two terminal monosaccharide residues were identified. KDO and galactose, which were typical for core structure of LPS isolated from bacteria grown at the higher temperature, were partially substituted by D-*glycero*-D-*talo*-octulosonic acid (ketooctonic acid, Ko) and D-*glycero*-D-*manno*-heptose, respectively [117].

It is noteworthy that the structure and composition of lipid A can determine involvement of different Toll-like receptors (TLR) during cell activation by endotoxins. Thus, LPS from *Acinetobacter baumannii* [118], *E. coli* [119], *S. minnesota* [120], *N. meningitidis* [4, 121], *B. henselae* [5], *B. multivorans* [55], and *F. tularensis* [122] activate the cells through the TLR4, whereas LPS from *L. pneumophila* [123], *Rhizobium* species Sin-1 [123], *C. trachomatis* LGV-1 [3], *P. aeruginosa* PAC-611, and *L. interrogans* signal through the TLR2 [3, 124]. Endotoxins from *B. fragilis* NCTC 9343 and *P. gingivalis* employ both receptors – TLR4 and TLR2 [3, 19, 125-128]. The TLR4-transfected CHO/CD14 cells (mutant cell line 7.7) responded to LPS from *E. coli* O111:B4, *F. nucleatum* ATCC 10953, and *Actinobacillus actinomycetemcomitans* Y4 (*A. actinomycetemcomitans*) by activation of the nuclear factor NF-κB, but did not respond to endotoxins from *P. gingivalis* 381 and *Capnocytophaga ochracea* 25 [129]. In this work it was also shown that LPS from *E. coli* O111:B4 (50 ng/ml) induced a significant release of IL-6 by cells of the human cell line U373 expressing TLR4 but not TLR2, whereas LPS from *P. gingivalis* 381 and *C. ochracea* 25 induced a minimal amount of this cytokine [129].

Core is a functionally important region of the LPS molecule connecting lipid A with O-polysaccharide. The minimal core structure (α-KDO-(2→8)-α-KDO-(2→4)-α-KDO-(2→6)-lipid A) consisting only of KDO residues was isolated from endotoxin of the wild strain *C. trachomatis* L2 [130].

The core region of enterobacterial LPS can formally be subdivided into an outer portion, distal from lipid A (proximal to the O-polysaccharide chain), and an inner portion directly linked to the lipid A. The complete outer core region (Ra-structure) mainly consists of hexoses and hexosamines, whereas inner core region is composed of KDO and heptose.

Comparison of outer and inner core structures of LPS from bacteria of the same genus revealed greater structural variability of the outer core in contrast to the inner core region. Thus, five outer core types (R1, R2, R3, R4, and K12) in LPS from different *E. coli* serotypes were earlier shown [131]. This classification is now confirmed by new examples of outer core structures in LPS isolated from the *E. coli* strains 2513 (R4) [17], F470 (R1), F576 (R2) [132], and F653 (R3) [17]. A new type of core was recognized in LPS from the *S. enterica* serovar Arizonae (O62). Its main difference from the known structures of the *Salmonella* core is the presence of an additional glucose residue instead of N-acetylglucosamine [133]. Two different core types are identified in LPS from *Klebsiella pneumoniae* [18, 30, 60] (Table 7). Core structures of endotoxins isolated from bacterial families Vibrionaceae, Pseudomonadaceae, and Rhodospirillaceae were described earlier [131]. The review article by Holst [137] covers the structural variety of LPS cores from different strains of *Proteus penneri*, *Serratia marcescens*, and three serovars of *Plesiomonas shigelloides* – O13, O54, and O74 [137].

The core regions of endotoxins from several Gram-negative bacteria include amino acids – alanine in LPS from *P. aeruginosa* immunotypes 1 and 5 [16, 31] and glycine in lipooligosaccharide (LOS) from *N. meningitidis*

Table 7. The structures of core regions of different bacterial endotoxins

Bacterium	Core structure	References
F. tularensis ATCC 29684*	Δ-GalNA-(1→3)-β-QuiNAc-(1→4)-[α-GalN-(1→2)]-β-Man-(1→4)-[β-Glc-(1→2)][α-Glc-(1→3)]-α-Man-(1→5)-α-KDO-(2→	[12]
H. pylori 26695	[O-polysaccharide→7)]-[α-Glc-(1→{-6)-α-Glc-(1-}$_{2\text{-}3}$→2)]-D-α-D-Hep-(1→2)-[α-Glc-(1→3)-α-Glc-(1→4)-β-Gal-(1→7)]-D-α-D-Hep-(1→2)-L-α-D-Hep-(1→3)-L-α-D-Hep7P-(1→5)-KDO-(2→	[134]
K. pneumoniae O3 (I type)	H/D-α-D-Hep-(1→4)-α-KDO-(2→6)-α-GlcN-(1→4)-α-GalA-(1→3)-[β-GalA-(1→7)-L-α-D-Hep-(1→7)]-L-α-D-Hep-(1→3)-[H/β-GalA-(1→6)-β-Glc-(1→4)]-L-α-D-Hep-(1→5)-[α-KDO-(2→4)]-α-KDO-(2→	[18]
K. pneumoniae 52145 (O1:K12) (II type)	α-GalA-(1→3)-[β-GalA-(1→7)-L-α-D-Hep-(1→7)]-L-α-D-Hep-(1→3)-[β-Glc-(1→4)]-L-α-D-Hep-(1→5)-[α-KDO-(2→4)]-α-KDO-(2→ or β-Glc-(1→6)-α-Glc-(1→4)-α-GlcNH$_2$-(1→4)-α-GalA-(1→3)-[β-GalA-(1→7)-L-α-D-Hep-(1→7)]-L-α-D-Hep-(1→3)-[β-Glc-(1→4)]-L-α-D-Hep-(1→5)-α-KDO-(2→	[30]
P. aeruginosa 2192	R→3)-L-α-D-Hep4,6P-(1→3)-L-α-D-Hep(Cm)2,4P-(1→5)-[α-KDO-(2→4)]-α-KDO-(2→, where R = α-Glc-(1→6)-[α-L-Rha-(1→3)]-β-Glc-(1→3)-[α-Glc-(1→4)]-α-GalN(L-Ala)-(1→ or α-Glc-(1→6)-β-Glc-(1→3)-[α-L-Rha-(1→6)-α-Glc-(1→4)]-α-GalN(L-Ala)-(1→	[32]
P. mirabilis R110/1959	D-α-D-Hep-(1→6)-α-GlcN-(1→4)-[D-α-D-Hep-(1→2)]-α-GalA-(1→3)-[β-GalA-(1→7)-L-α-D-Hep-(1→7)]-L-α-D-Hep6PEtNH$_2$-(1→3)-[β-Glc-(1→4)]-L-α-D-Hep-(1→5)-[β-L-Ara4N-(1→8)][α-KDO-(2→4)]-α-KDO-(2→	[26]
R. etli CE3	Fuc-Man-QuiNAc-KDO-(2→6)-α-Gal-(1→6)-[α-GalA-(1→4)]-α-Man-(1→5)-[α-GalA-(1→4)-[α-GalA-(1→5)]-KDO-(2→4)]-KDO-(2→	[135]
S. marcescens 111R	L-α-D-Hep-(1→2)-D-α-D-Hep-(1→2)-GalA-(1→3)-[L-α-D-Hep-(1→7)]-L-α-D-Hep-(1→3)-[β-Glc-(1→4)]-L-α-D-Hep-(1→5)-[Ko/KDO-(2→4)][β-L-Ara4N-(1→8)]-KDO-(2→	[136, 137]
S. pacifica KMM 3772	Glc-(1→2)-L-α-D-Hep-(1→6)-[L-α-D-Hep-(1→2)]-D-α-D-Hep7P(D-GroA)-(1→5)-KDO8N4R-(2→ R = P or PPEtNH$_2$	[48, 81]

* Sequence Δ-GalNA-(1→3)-β-QuiNAc is a modified fragment of the O-polysaccharide repeating unit.

(Tables 7 and 8). The biological role of amino acids existing in the core structure remains unclear.

The structural organization of LOS allows us to assign them to the group of intermediate molecules between typical R- and S-LPS structures. LOSs consist of lipid A and an oligosaccharide core. LOS are identified in such Gram-negative bacteria as *B. pertussis*, *N. meningitidis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae* [57], *Haemophilus ducreyi* (*H. ducreyi*) [140], *B. multivorans* [55], *Burkholderia* (*Pseudomonas*) *cenocepacia* [56], *A. addita* KMM 3600^T [53], and *Campylobacter jejuni* [138, 141] (Table 8). It is necessary to note that although *Y. pestis* is a member of the Enterobacteriaceae family, it differs from other enterobacteria by the absence of O-polysaccharide chain [117].

Core regions of LPS from *K. pneumoniae* and *H. pylori* as well as of LOS from *N. meningitidis*, *B. multivorans*, and *B. cenocepacia* are characterized by branched structure (Tables 7 and 8). LPS from *K. pneumoniae* R20 (O1$^-$:K20$^-$) has a very branched core with D,D-heptoglycan formed by heptose residues terminating the secondary chain of the core [60]. Such branching of the core oligosaccharide is formed by D-glucan linked to heptose IV residue in LPS from the *H. pylori* strain 26695 (Table 7). Subdivision of the core structure into α-, β-, and γ-chains is a specific feature of meningococcal LOS [139]. The considerable branching of the core structure of endotoxins from *N. meningitidis*, *K. pneumoniae*, *H. pylori*, *B. multivorans*, and *B. cenocepacia* influences molecular conformation and as a result may affect biological activity of the corresponding endotoxins [10, 142].

Outer parts of LOS core regions from *N. meningitidis*, *C. jejuni*, and *H. influenzae* are particularly interesting because they contain carbohydrate sequences identical to structures of membrane glycolipids from human cells [139-141]. This structural mimicry is well studied for LOS from *N. meningitidis* and *C. jejuni*. In the majority of *N. meningitidis* immunotypes (L2-L5 and L7) the core α-

Table 8. The structures of oligosaccharide fragments of LOS from different bacteria

Bacterium	Oligosaccharide structure	References
A. addita KMM 3600^T	α-Glc-(1-P→3)-L-α-D-Hep-(1→5)-α-KDO4P-(2→	[53]
B. cenocepacia ET-12 strain J2315	L-α-D-Hep-(1→7)-L-α-D-Hep-(1→7)-[α-L-Rha-(1→3)-β-D-QuiNAc-(1→7)-L-α-D-Hep-(1→2)-[α-D-Glc-(1→6)]-α-D-Gal-(1→3)]-L-α-D-Hep-(1→3)-[β-D-Glc-(1→4)]-L-α-D-Hep-(1→5)-[α-D-KoAra4N-(2→4)]-α-KDO-(2→	[56]
B. multivorans	β-D-QuiNAc-(1→3)-α-L-Rha-(1→2)-[β-D-Glc-(1→3)-α-D-GalNAc-(1→3)-β-D-GalNAc-(1→3)-α-L-Rha-(1→3)][L-α-D-Hep-(1→7)]-L-α-D-Hep-(1→3)-[L-α-D-Hep-(1→6)-β-D-Glc-(1→4)]-L-α-D-Hep-(1→5)-[α-D-Ko-(2→4)]-α-KDO-(2→	[55]
C. jejuni NCTC 11168	β-Gal-(1→3)-β-GalNAc-(1→4)-[α-Neu5Ac-(2→3)]-β-Gal-(1→3)-[α-Gal-(1→2)]-β-Gal-(1→3)-[β-Glc-(1→2)]-L-α-D-Hep-(1→3)-[β-Glc-(1→4)]-L-α-D-Hep6P/PEtNH$_2$-(1→5)-KDO-(2→	[138]
N. meningitidis L2	α-Neu5Ac-(2→3)-β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-Glc-(1→4)-[α-GlcNAc-(1→2)-[α-Glc-(1→3)]-L-α-D-Hep6PEtNH$_2$(7PEtNH$_2$/Gly)-(1→3)]-L-α-D-Hep-(1→5)-[α-KDO-(2→4)]-KDO-(2→	[139]

chain includes a carbohydrate constituent, lacto-N-neotetraose β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-β-Glc-(1→ [139]. This tetrasaccharide is identical to the carbohydrate sequence of paragloboside (lacto-neotetraosyl ceramide) from human erythrocyte membrane [143]. LOS from *N. meningitidis* immunotypes L2-L5 also includes lacto-N-neotetraose, but in this case it is masked by sialic acid linked to the terminal galactopyranosyl residue leading to pentasaccharide (sialoparagloboside), which also has been found among gangliosides of human erythrocyte membrane [144]. LOS from *N. meningitidis* immunotype L1 is structurally related to the P^k-antigen of human erythrocytes – α-Gal-(1→4)-β-Gal-(1→4)-β-Glc-(1→ [143]. The core outer part of LOS from the *C. jejuni* serogroups O:19 and O:4 contains the terminal pentasaccharide of the ganglioside GD$_{1a}$ (α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GalNAc-(1→[α-Neu5Ac-(2→3)]4)-β-Gal-(1→) and a tetrasaccharide epitope identical to the ganglioside GM$_1$: β-Gal-(1→3)-β-GalNAc-(1→[α-Neu5Ac-(2→3)]4)-β-Gal-(1→ [145, 146]. Mimicry to tetra- and trisaccharide sequences of the ganglioside GM$_2$ was found in the cores of LOS from the *C. jejuni* serotypes O:1, O:23, and O:36 [141, 145] and of LOS from the *C. jejuni* strain NCTC 11168 also. The core structure of the latter strain has an additional homology to ganglioside GM$_{1a}$ (Table 8). LOS possessing sialic acid residues can interact with specific receptors, Siglecs (immunoglobulin-like lectins), expressed on the surface of monocytes/macrophages and natural killer cells [147-149].

Comparison of core regions from different bacterial endotoxins has revealed that the sequence L-α-D-Hep-(1→3)-L-α-D-Hep-(1→5)-KDO-(2→6)-lipid A is a typical structural component of the inner core region in the majority of LPS (Tables 7 and 8). Six variants of the core structure containing this carbohydrate sequence are identified in endotoxins from *Y. pestis* cultivated at different temperatures [117]. The complete absence of heptose within the core region is specific for LPS from *R. etli* CE3 [135], *Rb. sphaeroides* ATCC 17023 [131], and *F. tularensis* ATCC 29684 [12] (Table 7). The structure of LPS from the phototrophic bacterium *Rb. capsulatus* is not completely determined. In addition to galactose and glucose, the described core region of LPS from this bacterium contains sialic acid residue [131, 150].

A successive truncation of Ra-structure LPS associated with specific alterations of core oligosaccharide biosynthesis in different *Salmonella* strains (R-mutants) results, respectively, in the Rb, Rc, Rd, and Re core structures. The last structure, which contains only lipid A and KDO residues, is a minimal LPS structure. Core structures of endotoxins isolated from the *Salmonella* R-mutants can be more precisely classified in accordance with the number of heptose and hexose residues (Rd$_1$ and Rd$_2$, Rb$_1$-Rb$_4$) or of phosphate groups (Rd$_1$P$^+$ and Rd$_1$P$^-$) within the core region [131].

LOS from *B. multivorans* and *B. cenocepacia* are characterized by the presence within the core region of D-*glycero*-D-*talo*-octulosonic acid (Ko) residue whose negative charge is compensated by the presence of 4-amino-4-deoxy-L-arabinose (Table 8). This acidic component was also identified in endotoxins isolated from *S. marcescens* [136] and *Y. pestis* strains cultivated at 25°C [117, 151]. In contrast to endotoxins from members of *Burkholderia*, *Yersinia*, and *Serratia* genera, in LPS from *Acinetobacter haemolyticus* NCTC 10305 the core region is connected with the lipid A through glycosidic linkage of Ko instead of KDO [56, 152]. Endotoxins from the marine bacteria *Shewanella algae* BrY, *S. oneidensis* MR-1, and *S. pacifica* KMM 3772 are shown to have 8-

amino-3,8-dideoxy-D-*manno*-octulosonic acid residue (KDO8N) as a component connecting the core oligosaccharide with lipid A. In the core region of endotoxins from the last two bacteria the phosphate or 2-aminoethylpyrophosphate groups can be linked to position 4 of the 8-amino-3,8-dideoxy-D-*manno*-octulosonic acid residue [48, 81, 153, 154]. Phosphorylated KDO residue is a specific structural feature of the core region of endotoxins from *V. cholerae* and *H. influenzae* [155] and also endotoxins from the marine bacteria *Pseudoalteromonas carrageenovora* IAM 12662^T, *P. issachenkonii* KMM 3549^T, and *A. addita* KMM 3600^T [48, 53, 70, 156]. An unusual location of KDO was revealed in LPS from *K. pneumoniae* O3, O4, and O12 [157], *R. etli* [135], and *Proteus vulgaris* O25 [33] (Table 7). For example, KDO was detected as terminal residue at the non-reducing end of O-polysaccharide chain in endotoxins from *K. pneumoniae* O4 and O12 [157]. When located within polysaccharide portion of endotoxin, KDO can be connecting residue between the core region and the O-polysaccharide chain [158].

Studies on R-LPS with different length of core region revealed influence of the core structure extension on endotoxin internalization by human phagocytic cells. Thus, kinetics of internalization of different LPS chemotypes from *E. coli* by human peripheral blood mononuclear cells correlated with the increase in the core region length and as a consequence with the decrease in the molecule hydrophobicity [159]. Incubation of the cells with endotoxins for 1 h resulted in internalization of up to 70% of the bound Ra-LPS, whereas for the isolated lipid A this value was only 30%. Note that the internalization of Re-LPS was observed only during the second hour of the incubation (10%) [159].

The role of the core composition in the biological activity of LPS was shown in many works. The TNF-α release by neutrophils induced by different endotoxin structures decreased in the following order: Ra-LPS from *E. coli* K12 > Rd_1P^--LPS from *S. minnesota* R7 > Re-LPS from *E. coli* F515 > lipid A from *E. coli* F515 [159]. Studies on the influence of different LPS chemotypes from *E. coli* on production of reactive oxygen species by human neutrophils indicated that endotoxins caused significant decrease in the luminol-dependent chemiluminescence in the following order: S-LPS > Ra-LPS > Re-LPS > Rc-LPS > control. In this work, the dependence of inhibition of human neutrophil apoptosis caused by *E. coli* LPS on the bacterial chemotype also shown. The maximal inhibition was observed for Re-LPS consisting of lipid A and two KDO residues only [160]. Interaction of R-LPS from *E. coli* with bovine neutrophils activated IL-8 and TNF-α secretion, but IL-1β release was not observed. Ra-LPS from *E. coli*, unlike Rd-LPS from *E. coli*, increased secretion of both IL-8 and TNF-α [161].

KDO residues not only play the structural function connecting the core region with the lipid A, [47] but are important constituents of LPS biological activity as well. Thus, a synthetic compound consisting of lipid A with two KDO residues exhibited a higher biological activity than similar structures with one KDO residue or without it [102, 162, 163]. Removal of $(KDO)_2$ through a mild acidic hydrolysis [164] decreased the meningococcal LOS biological activity [4]. It seems that KDO glycosylation of lipid A can influence the mobility of lipid A fatty acid residues and lipid A conformation and as a result – LPS endotoxic activity [6].

In addition to the most widely distributed phosphate groups and KDO, the core region of endotoxins includes pyrophosphate, 2-aminoethylphosphate, and 2-aminoethylpyrophosphate groups, 4-amino-4-deoxy-L-arabinose (*P. mirabilis* R45, *Y. pestis*) and uronic acid residues, which also determine the charge of core region. A minor decrease in the total negative charge of the LPS can occur if an extended O-polysaccharide chain presents in the LPS composition. For example, the charge of the S-LPS from *S. typhimurium* strain SL3770 is approximately −7.3, whereas for Ra-LPS from the *S. typhimurium* SL3749 it reaches −8.2 [165]. The total charge of S-LPS from *Chromobacterium violaceum* and S-LPS from *Rb. capsulatus* 37b4 is estimated as $\geqslant -4.0$ and $\geqslant -4.5$, respectively. Charges of endotoxins from a wild strain of *S. minnesota* and mutant Ra-LPS from *S. minnesota* R60 and Rb_1-LPS from *S. minnesota* R345 with a full-value structure of the inner core are estimated as $\geqslant -5.0$. Rc-LPS from *E. coli* J-5 has a similar negative charge [166]. A significant overall charge (−7.0) is determined for the R-LPS from *K. pneumoniae* R20 whose endotoxins contain D-galacturonic acid residues in the core region [60, 166]. It seems that the core region of LPS from *P. aeruginosa* 2192 containing four phosphate groups and two KDO residues is the most acidic [32].

The presence of ethanolamine and/or 4-amino-4-deoxy-L-arabinose in the inner core region or lipid A compensates the negative charge of phosphate groups and significantly decreases the charge of a LPS. Thus, the charge of Rb_2-LPS from the *S. typhimurium* strain SH5357 is −4.5 whereas the charge of Rb_2-LPS from the strain SH5014 is −7.4. This explains the higher resistance of the *S. typhimurium* SH5357 to the cationic antibiotic polymyxin B (PMB) relative to the sensitive strain SH5014 [165]. Charges of Rc-LPS from *S. typhimurium* HN202 and of Re-LPS from *S. minnesota* R595 are estimated as −7.8 and −3.4, respectively [63, 165, 166]. Influence of the charge density of the bilayer surface with the outer monolayer formed by Re-LPS from *E. coli* F515 (−4.0) or *P. mirabilis* R45 (−3.0) on interaction with PMB was shown in work [63]. The authors found that the absence in the inner core region and lipid A of cationic groups of ethanolamine and 4-amino-4-deoxy-L-arabinose resulted in increased number of PMB molecules bound to the bilayer surface. Data obtained by us on LPS release from the outer membrane

of Ra- and Re-chemotypes of *E. coli* D21 and *E. coli* JM103, respectively, under the influence the cationic protein lactoferrin are consistent with these findings [167, 168]. The negative charge of the lipid A in the cell wall of *E. coli* strain D21 is shielded by a relatively longer core oligosaccharide that develops increased resistance of this bacterial strain to interaction with this cationic protein in comparison to its other chemotype (*E. coli* JM103) [167, 168].

Functionally active groups of the core region are involved in the endotoxin interaction with blood proteins and in expression of biological activity by LPS. As it was mentioned above, the absence of negatively charged KDO residues decreased the meningococcal LOS biological activity [4]. Interaction of complement component C4b with a 2-aminoethylphosphate group at O-6 position of heptose II of *N. meningitidis* LOS was more efficient than with this group at O-3 position of the same heptose [169]. This functional group is linked to O-6 position of heptose II in the inner core of endotoxins from *N. meningitidis* immunotypes L2 and L4 [139] and endotoxins from *P. mirabilis* R110/1959 (Table 7). In the majority of *C. jejuni* serogroups, the 2-aminoethylphosphate group is linked to heptose I [138, 141].

The role of the core individual monosaccharides in phagocytosis of bacteria and their resistance to serum proteins is now clearer. In particular, *E. coli* K12 180 and *Y. pestis* KIM-6 belonging to R-chemotype can occupy human alveolar macrophages better than the same bacteria of the S-chemotype [170]. CD209 transfected HeLa cells phagocytized *E. coli* Ra-chemotype but not S-chemotype or deep R-mutants [171]. Bacteria *H. pylori* and certain strains of *K. pneumoniae* with LPS possessing the Le^X-structure or mannose residues interact with the CD209-expressing cells, in particular, with human alveolar macrophages [171-173]. This receptor is a C-type lectin and belongs to the mannose-recognizing receptor family [170, 174]. The greatest binding of CD209 was observed with oligosaccharides including a fucosylated or mannosylated N-acetylglucosamine residue [174]. The authors supposed that this disaccharide should play an essential role in the interaction of LOS/LPS with CD209. This was confirmed with mutant strains of *E. coli*, *N. gonorrhoeae*, *S. typhimurium*, and *H. ducreyi* unable to express *N*-acetylglucosamine unit within the outer core region of LOS/LPS. Removal of this constituent decreased the receptor CD209-mediated interaction of the bacteria with HeLa cells [174]. The similar role of the bacterial chemotype in the interaction with serum proteins was shown in work [175]. The data presented suggested that the composition of outer core region (Ra-structure of LPS) and LOS could play an important role in phagocytosis of Gram-negative bacteria with involvement of cell CD209 receptors. The outer core epitopes needed for the interaction with the receptors can be masked by the presence of O-polysaccharide chain, and this is a factor of bacterial resistance to action of serum proteins and phagocytosis [176, 177].

The data presented indicate that the core region is a functionally important constituent of LPS, and its composition and structure influence the biological activity of endotoxins.

O-Polysaccharide or the O-specific chain is a polysaccharide that includes from one (SR-LPS) to 25 chemically identical repeating oligosaccharide units (S-LPS), which in turn include from 2 to 7 monosaccharide residues (Fig. 2) [178].

To distinguish the antigenic specificity of O-polysaccharide among other bacterial surface antigens (K, H), it is termed somatic antigen (O-antigen). Being isolated from lipid A, O-antigen does not display a typical endotoxic activity as is inherent in lipid A [57]. To establish the composition of O-polysaccharides, they are subjected to complete acidic hydrolysis that releases monosaccharide components [180]. Structure of the repeating unit of O-polysaccharide (the chemical repeating unit) is established using chemical approaches and NMR spectroscopy. This structure can be identical to or different from the structure of the so-called biological repeating unit produced during O-polysaccharide biosynthesis (Fig. 2). Determination of the oligosaccharide structure isolated from SR-LPS allows the structure of the biological unit (O-unit) of O-polysaccharide to be established. At present, structures of O-units are established for LPS from *E. coli* O6, O91, and O126 [181, 182, 183]; *Hafnia alvei* [34]; *P. shigelloides* O54:H2 and O74:H5 [35, 36]; and *S. enterica* serovar Toucra O48 [13]. Chemical compositions of O-units of LPS from all *P. aeruginosa* serotypes are characterized most completely [16, 31, 184]. Structure of the LPS O-unit plays essential role in the immunological specificity of bacterial strains, which strongly depends on the particular chemical nature of monosaccharide located at the non-reducing terminus of the O-polysaccharide chain [34].

The O-specific chain can be a linear or a branched polysaccharide as in the case of LPS from *E. coli* O158 [178, 185]. Most often O-antigen is a heteropolymer [178, 186]. A homopolymeric O-specific chain was found in LPS only from some bacterial serogroups such as *E. coli* O8 and O9, *K. pneumoniae* O3 and O5, *L. pneumophila*, *Salmonella* T1, *Y. rohdei* WA339, *B. bronchiseptica*, *B. parapertussis*, and *Vibrio cholerae* O1 (the Hakata and Inaba strains) [186]. The structure of repeating units, namely, the monosaccharide nature and ring form (pyranosyl or furanosyl), location and configuration of the O-glycoside bond (α or β), as well as the presence of substituents are different among the strains and determine the bacterial serotype [46]. The O-antigen possesses a specific immunological activity [187]. The majority of *P. aeruginosa* strains possess two types of LPS with structurally different polysaccharide chains termed A- and B-band polysaccharides [188, 189].

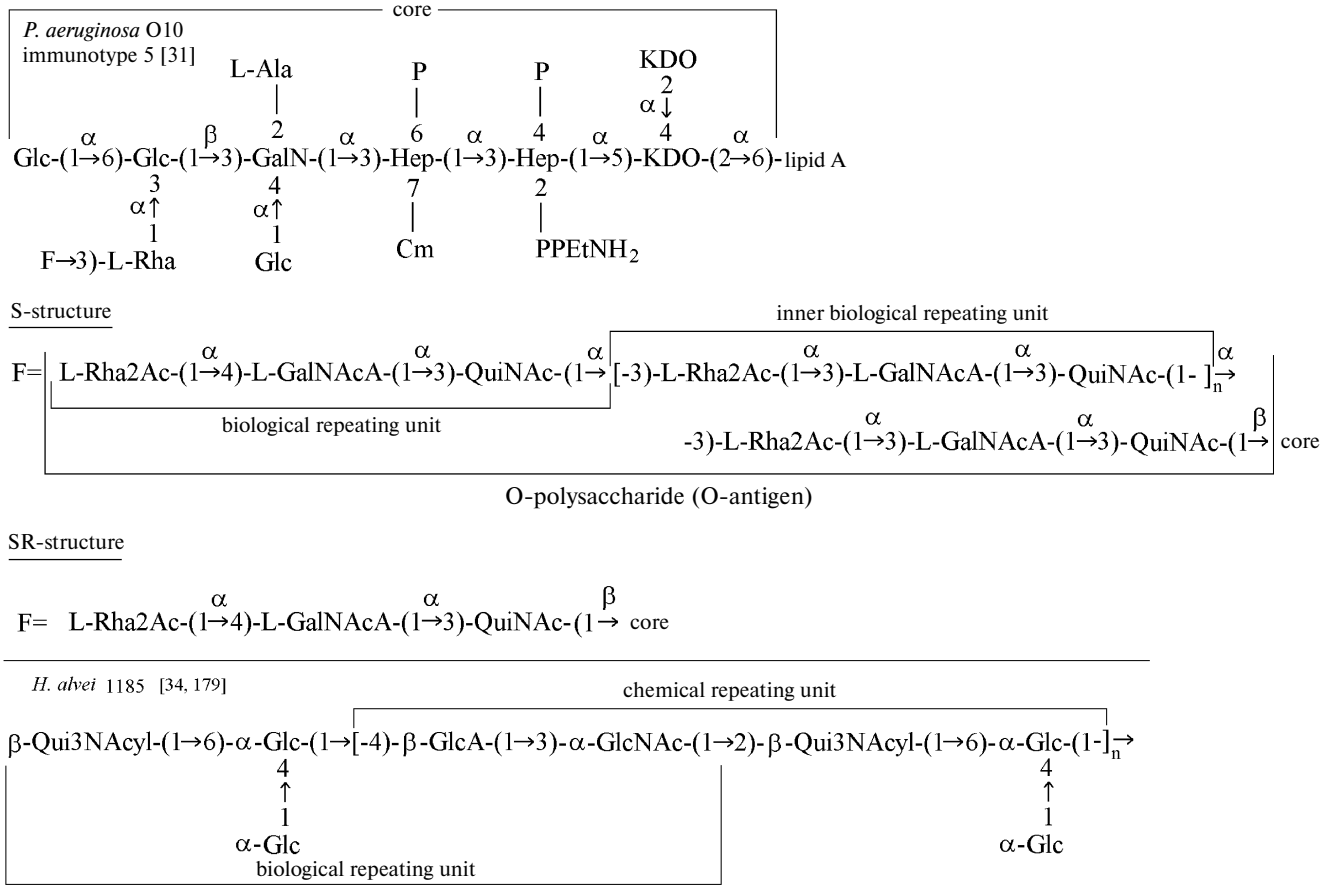

Fig. 2. Examples of chemical and biological repeating units of S- and SR-LPS.

The number of identified components of O-antigens is large and constantly increasing [13-15, 37-43]. Structures of the chemical repeating unit of O-polysaccharide in different bacterial genera such as *Salmonella*, *Shigella*, *Escherichia*, *Klebsiella*, *Yersinia*, *Proteus*, *Pseudomonas*, etc., are presented in detail in a review [186]. Most complete structures of the repeating unit of virtually all identified *E. coli* serotypes are presented in review [178]. The review [48] comprises the composition and structure of O-polysaccharide and also of the core and lipid A of endotoxins from marine bacteria of the genera *Alteromonas*, *Pseudoalteromonas*, and *Shewanella*.

O-Antigens of the great majority of *Klebsiella* species are neutral polymers [166]. Table 9 presents structures of the chemical repeating unit of neutral O-antigens of LPS from *P. mirabilis* O20 and the *H. pylori* strain 26695. This group also includes a homopolymeric A-band O-polysaccharide of LPS from the *P. aeruginosa* PAO1 (A^+B^+) composed by D-rhamnose [196].

Several O-antigens include carbohydrate sequences similar to human blood Lewis-antigens. For the first time the structural similarity of the Le^d antigen and the linear trisaccharide fragment of the O-specific chain was shown for endotoxin from *Salmonella arizonae* O45 [197]. The structures of Le^X, {-3)-β-Gal-(1→[α-L-Fuc-(1→3)]4)-β-GlcNAc-(1-}, and of Le^Y, {α-L-Fuc-(1→2)-β-Gal-(1→[α-L-Fuc-(1→3)]4)-β-GlcNAc-(1-}, blood antigens are mainly found in O-antigens of LPS from *H. pylori* [157, 198, 199].

The role of the chemical structure of O-antigens in the interaction of LPS with membranes of target cells is still unclear. Comparison of the number of negatively charged groups within a structural chemical unit allows us to assess the difference in acidity of O-antigens. As a rule, concentration of negative charges in a repeating structural unit is not higher than one acidic function per three monosaccharide residues [186]. This ratio of acidic to neutral monosaccharides is observed in O-antigens from the *E. coli* serogroups O24, O56, O58, O104, and O138 [186, 191] (Table 9). The ratio 1 : 4 of acidic monosaccharides to neutral ones is found in the repeating unit of O-polysaccharides from *E. coli* serogroups O22, O83, O113, O116, O141, and O159 [178, 192]. The highest negative charge was detected in O-antigens of LPS from the *B. bronchiseptica* strains 110H and 512 as well as from *B. parapertussis* formed only by 2,3-diacetamido-2,3-dideoxy-L-galacturonic acid [190], and from *P. mirabilis* O13 [41]. The charge of the last polysaccharide is deter-

Table 9. The structures of chemical repeating units of different bacterial O-antigens

Bacterium	Structure of repeating unit	References
B. bronchiseptica 110H and Bp5132	[→4)-α-L-GalNAc3NAcA-(1→]$_n$	[190]
E. coli O56	[→7)-α-Neu5Ac-(2→3)-[α-Gal-(1→2)]-β-Glc-(1→3)-β-GlcNAc-(1→]$_n$	[191]
E. coli O104	[→3)-β-GalNAc-(1→4)-α-Gal-(1→4)-α-Neu5,7,9Ac$_3$-(2→3)-β-Gal-(1→]$_n$	[186]
E. coli O159	[→3)-[α-L-Fuc-(1→4)]-β-GlcNAc-(1→4)-α-GalA-(1→3)-α-L-Fuc-(1→3)-β-GlcNAc-(1→]$_n$	[192]
H. pylori 26695*	α-L-Fuc-(1→2)-β-Gal-(1→4)-[α-L-Fuc-(1→3)]-β-GlcNAc-(1-[→3)-β-Gal-(1→4)-[α-L-Fuc-(1→3)]-β-GlcNAc-(1-]$_n$→3)-D-α-D-Hep-(1→2)-D-α-D-Hep-(1→6)-D-α-D-Hep-(1→	[134]
K. pneumoniae O4*	α-KDO-(2→2)-β-Rib*f*-(1-[→4)-α-Gal-(1→2)-β-Rib*f*-(1-]$_n$→4)-α-Gal-(1→	[157]
K. pneumoniae O12*	β-KDO-(2→3)-α-L-Rha-(1→[-3)-β-GlcNAc-(1→4)-α-L-Rha-(1→]$_n$	[157]
P. mirabilis G1	[→3)-β-GalNAc-(1→6)-[α-GalA6(L-Lys)-(1→4)]-β-GalNAc-(1→4)-β-GlcA-(1→]$_n$	[193]
P. mirabilis D52	[→2)-[D-Rib-ol-(5-P→3)]-β-Gal-(1→3)-α-GlcNAc-(1→3)-β-Glc6PEtNH$_2$-(1→3)-β-GlcNAc-(1→]$_n$	[14]
P. mirabilis O13	[→3)-β-GlcNAc-(1→3)-[α-GalA6(AlaLys)-(1→4)]-α-Gal-(1→]$_n$	[41]
P. mirabilis O20	[→3)-[α-Glc-(1→2)-β-Gal-(1→4)]-α-GlcNAc-(1→4)-β-Glc-(1→3)-β-GlcNAc-(1→]$_n$	[15]
P. mirabilis O27	[→3)-α-GalA6(L-Ala)-(1→3)-β-GlcNAc6PEtNH$_2$-(1→3)-[β-GlcNAc-(1→4)]-β-GlcA6(L-Lys)-(1→]$_n$	[194]
P. aeruginosa 170041	[→2)-α-L-Rha-(1→4)-α-GalNAcAN3Ac-(1→4)-α-GalNFoAN-(1→3)-α-QuiNAc-(1→]$_n$	[16]
R. etli CE3*	α-Fuc2,3,4Me$_3$-(1-[→4)-α-GlcA-(1→4)-α-6dTal3Me-(1→3)-α-L-Fuc-(1-]$_n$→3)-α-L-Fuc-(1→3)-β-Man-(1→3)-β-QuiNAc-(1→4)-α-KDO-(2→	[195]
S. toucra O48	[→4)-α-Neu5Ac-(2→3)-α-L-FucNAc-(1→3)-β-GlcNAc-(1→]$_n$	[13]

* Structures of LPS O-antigen include biological repeating units.

mined by an unusual acid N$^{\varepsilon}$-[(*R*)-1-carboxyethyl]-L-lysine that has two free carboxyl groups. The presence of KDO at the reducing or non-reducing end of the polysaccharide determines the charge of O-antigens from *K. pneumoniae* O4 and O12 [157] and *R. etli* CE3 [195].

A separate group is represented by O-antigens that contain saccharides with functionally active residues (e.g. acetamidine residue, amino group of ethanolamine, and/or ε-amino group of lysine). O-Antigens of the bacteria *P. mirabilis* G1, O27, and O28 [14, 41, 193, 194] are distinguished by the presence of single amino acid residues, such as those of L-lysine, L-alanine, or L-serine (Table 9). In some O-antigens of *P. aeruginosa* the negative charge of uronic acid residues is neutralized partially (the O6a and O6a,6c serotypes) or completely (the immunotype 1, the O6a,6b and O6a,6d serogroups) [16]. The negative charge of O-antigens can be compensated by the presence in the polymer of the acetamidine group [186], as it occurs in O-polysaccharides of *P. aeruginosa* (immunotypes 3, 4, and 7). The B-band O-polysaccharide prepared from the *P. aeruginosa* strain PAO1 (A^+B^+) and from the *P. aeruginosa* mutant strain dps89 (A^-B^+) consists of repeating trisaccharide units formed by 2-acetamido-2,6-dideoxy-D-galactose (N-acetyl-D-fucosamine), 2,3-diacetamido-2,3-dideoxy-D-mannuronic, and 3-acetamidino-2-acetamido-2,3-dideoxy-D-mannuronic acid residues [196, 200]. O-Antigen of *P. mirabilis* D52 is characterized by the presence of a phosphorylated ribitol residue [14].

High content of deoxysaccharides was found in O-antigen of LPS from *F. tularensis* 15 [186]. The most hydrophobic O-polysaccharide was detected in LPS from *L. pneumophila* (*Philadelphia*). It is homopolymer consisting of 5-acetamidino-7-acetamido-8-*O*-acetyl-

3,5,7,9-tetradeoxy-D-*glycero*-D-*galacto*-nonulosonic acid residues [186]. Despite a high negative charge of O-antigen from LPS of *L. pneumophila*, it does not form stable complexes with CD14 [96].

Significant role of the O-antigen structure in LPS biological activity was confirmed by activation of the complement system through the way similar to the "lectin pathway". Endotoxins containing O-polysaccharide enriched with D-mannose or N-acetylglucosamine residues are supposed to be more active than other known structures lacking these sugars [201]. O-Antigens of *K. pneumoniae* O3 and O5 and also of *E. coli* O8 and O9 include only D-mannose residues [186].

The presence of a polysaccharide component in endotoxin from *Salmonella* is essential for activation of human cells [11]. The lipid A isolated from Re-LPS of *S. minnesota* R595 and the synthetic compound 516 did not stimulate activity of the nuclear factor NF-κB in differentiated human THP-1 cells in the presence of 10% serum, up to the endotoxin concentration of 1 μg/ml. In contrast, compound 506, S-LPS from *E. coli* O111:B4, and the lipid A from *E. coli* F583 displayed a significant activity even at the concentration of 10 ng/ml [11]. These data are supplemented by studies of Tanamoto et al. [9, 25]. The TNF-α release by differentiated THP-1 cells upon their interaction with S-LPS from *S. minnesota*, *S. typhimurium* LT2, or *Salmonella abortus equi* in the presence of serum (10%) was higher than that with isolated lipids A and compound 516 [9, 25, 103]. In addition [202], the agonistic activity of S-LPS from *Salmonella* in activation of the TNF-α synthesis in human whole blood decreased as follows: S-LPS from *S. enteritidis* > S-LPS from *S. typhimurium* > S-LPS from *S. minnesota* > Re-LPS from *S. minnesota* R595. It seems that for the S-LPS from the same bacterial genus the length of the O-polysaccharide chain of LPS influences the endotoxin biological activity.

S-LPS from different bacteria can differ in their ability to stimulate cells for producing different cytokines. For example, S-LPS from *K. pneumoniae* stimulated synthesis of IL-10 in human whole blood more actively than S-LPS from *E. coli* or from *S. typhosa*. On the other hand, the two last endotoxins induced synthesis of TNF-α, INF-γ, and IL-1β more actively than S-LPS from *K. pneumoniae* [203]. Note that S-LPS from *S. marcescens*, which contains bisphosphorylated pentaacyl lipid A, induced synthesis of TNF-α similarly to S-LPS from *S. typhimurium* [68, 202]. LOS from *N. meningitidis* was the strongest agonist in inducing TNF-α synthesis by differentiated THP-1 cells: LOS from *N. meningitidis* > S-LPS from *V. cholerae* > S-LPS from *E. coli* > S-LPS from *P. aeruginosa* > S-LPS from *K. pneumoniae* > Ra-LPS from *S. typhimurium* > Re-LPS from *S. minnesota* [10].

The role of O-polysaccharide in the LPS interaction with target cells and blood proteins was shown in some works [11, 204-206]. LPS can activate cells *in vitro* in the absence of serum and, consequently, without involvement of LBP and sCD14 proteins [207-209]. Thus, the IL-1 release from human mononuclear cells stimulated by different glycoforms of LPS (100 ng/ml) in the absence of serum decreased in the following order: S-LPS from *S. abortus equi* > Rb_2-LPS from *S. minnesota* R345 > Re-LPS from *S. minnesota* R595 > Rd_1P^--LPS from *S. minnesota* R7 [84]. In the absence of serum, S-LPS from *S. minnesota* 6261 was the more potent agonist of the TNF-α synthesis by human monocytes than Re-LPS from *S. minnesota* R595 [207]. These data suggest that in the absence of serum LPS containing O-polysaccharide chain are more active agonists for human cells than structural variants of LPS lacking O-polysaccharide or possessing a significantly truncated core region. However, addition of serum increased cell activation by endotoxins [210]. Depending on the LPS structure and concentration, serum could modulate TNF-α induction by both human and mouse responsive cells (WEHI-3) [207, 208]. Thus, the maximal TNF-α release by human monocytes stimulated by S-LPS from *S. minnesota* 6261 was obtained in the presence of 0.1% serum, whereas for the same response to Re-LPS from *S. minnesota* R595 at least 10% serum was required [207]. At this concentration of serum, the activity of Re-LPS from *S. minnesota* R595 was higher than the activity of S-LPS from *S. minnesota* 6261. At concentration of S-LPS from *S. minnesota* 6261 higher than 10 ng/ml, the amount of TNF-α released into the medium was comparable to its amount released in the absence of serum. In the case of Re-LPS from *S. minnesota* R595, the addition of serum to the medium significantly increased the TNF-α release at every concentration of this endotoxin. Substitution of serum by rLBP increased 10-100 times the TNF-α release upon cell activation by S-LPS from *S. minnesota* 6261 and nearly 10-fold decreased TNF-α release upon activation by Re-LPS from *S. minnesota* R595. The presence of only rsCD14 was also more significant for S-LPS from *S. minnesota* 6261 (TNF-α production was increased 5-10-fold) than for Re-LPS from *S. minnesota* R595. These results indicated that the helper activities of rLBP and rsCD14 were more pronounced when the cells were activated by S-LPS rather than with Re-LPS [207]. The activities of S- and Re-LPS observed with the increase in the serum concentration can be explained by the critical aggregation concentration values – this value is significantly higher for S-LPS than for Re-LPS [211]. The higher affinity of S-LPS (compare to R-LPS) for the CD14 binding was confirmed by several studies [204-206].

From the data obtained with macrophages of Heedless mice ($CD14^{-/-}$), it was supposed that in the absence of mCD14 the receptor complex TLR4/MD-2 should recognize different LPS glycoforms, whereas the presence of mCD14 should abolish this ability [212]. Thus, the MyD88-dependent signaling pathway in

$CD14^{-/-}$ mouse macrophages was activated only in response to Re-LPS or the lipid A from *S. minnesota* R595 but not upon cell stimulation with S-LPS from *Salmonella* [212]. The absence of CD14 and LBP limited the activity of S-LPS from *E. coli* and *Salmonella*, whereas activation of mouse cells expressing TLR4/MD-2 by R-LPS did not depend on the presence of these proteins [213]. These results about the activation of the MyD88-dependent and MyD88-independent signalization by different LPS glycoforms are different from those obtained with the human THP-1 cell line. In the presence of mCD14, S-LPS from *E. coli* O55:B5 or from *V. cholerae* Inaba 569B selectively activated the MyD88-dependent signaling pathway, whereas Ra- and Re-LPS from *Salmonella* activated the MyD88-independent signalization. LOS from *N. meningitidis* stimulated activation of both signaling pathways [10]. It is possible that the ability of this LOS to stimulate synthesis of both MyD88-dependent mediators (TNF-α, IL-1β, MCP-1, and MIP-3α) as well as MyD88-independent ones (INF-β and nitrogen oxide) is an explanation of its highest agonistic activity among endotoxins studied to date. Note that the hexaacyl lipid A from *N. meningitidis* with symmetrically distributed fatty acids used in this work is different from the lipid A of *V. cholerae* by the increased content of C12-fatty acid residues only [10].

An LPS-like extract (CyP) from the cyanobacterium *Oscillatoria planktothrix* FP1 has been shown to inhibit the MyD88-dependent and MyD88-independent activation in the myeloid cell line. CyP significantly inhibited the activation by S-LPS from *E. coli* or from *S. abortus equi* of dendritic cells (DC) obtained by differentiation of human monocytes. In addition, CyP did not activate directly the genes encoding cytokines such as TNF-α, IL-6, and IL-10 in these cells. Therefore, the authors suggested that CyP should be an antagonist of endotoxins on the TLR4/MD-2 level. Note that CyP decreases production of mediators even after cell stimulation by LPS. Thus, addition of CyP to the LPS-stimulated DC cells at different times of incubation (on the 3rd, 6th, and 9th hour) pronouncedly decreased the number of transcripts for TNF-α and IL-6 [214]. CyP also inhibited the secretion of TNF-α, IL-1β, and IL-6 induced by *N. meningitidis* MC58 LOS in human whole blood. However, at high concentration (20 μg/ml) CyP itself stimulated the secretion of IL-8 and MCP-1 chemokines in human whole blood [86]. In addition to long chain saturated and unsaturated fatty acid residues, the structure of cyanobacterial LPS-like molecules is characterized by low content of KDO and absence of L-*glycero*-D-*manno*-heptose residues [214].

The agonistic activity of endotoxins, as has been recently shown, depends on the presence of a full structure of core region as well as O-polysaccharide chain in comparison with different structures of isolated lipid A [92]. On using S-LPS from *Shigella flexneri* 2a, which is a mixture of hexa-, penta-, and mainly tetraacylated structures of the lipid A and S-LPS from *E. coli* K235 (hexaacylated lipid A), the first preparation possessed lower agonistic activity in the activation of nuclear factor NF-κB in human HEK293T cells transfected with CD14/TLR4/MD-2 receptor complex than the endotoxin from *E. coli* K235. A similar result was also obtained by the authors with human monocytes. Synthetic compounds hexaacylated 506 and pentaacylated LA-21-PP (up to concentration 8 μg/ml) did not activate transfected HEK293T cells. Compound 506 revealed agonistic activity only when the concentration of vector MD-2 expression was increased up to 100-fold, whereas compound LA-21-PP remained inactive [92]. Thus, an increase in the MD-2 expression promoted the activation of CD14/TLR4/MD-2 cells by the hexaacylated structure of lipid A.

At high LBP concentration in blood and the presence of O-polysaccharide within LPS structure determine the preferential transfer of S-LPS to plasma lipoproteins as compared to the transfer of R-LPS [210]. This is explained by the strict dependence of the S-LPS interaction with the cells on CD14 presence, whereas the interaction of R-LPS is partially independent of this receptor. High concentrations of LBP inhibited the binding of the soluble protein sCD14 with both S- and R-LPS [210].

From the data obtained by us about S-LPS *E. coli* O55:B5 influence on human erythrocyte electrophoretic mobility (EPM) values, we concluded that the presence of a long O-polysaccharide chain within the LPS structure can mask the cell surface charge upon LPS insertion into erythrocyte membranes [215]. This can result in cell aggregation and thrombogenesis under conditions of endotoxin shock caused by Gram-negative bacteria. Ra- and Rc-structures of LPS from *E. coli* had virtually no influence on the average EPM value of sensitized erythrocytes as compared to the control cells [215]. Incubation of human erythrocytes with antagonistic SR-LPS from *Rb. capsulatus* insignificantly decreased the EPM average value of the cells (in the range of 2-4%) that could be associated with the absence of a long polysaccharide chain in the structure of studied LPS [216].

Data presented in this review confirm the role of all structural elements of lipopolysaccharides in their biological activity.

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