STRUCTURE AND PROPERTIES OF LIPID A – A COMPONENT OF GRAM-NEGATIVE BACTERIA

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A review is given of literature information on the structure, biosynthesis, and biological properties of lipid A, the hydrophobic section of the endotoxins of Gram-negative bacteria.

Lipid A is the hydrophobic section of the lipopolysaccharides (LPSs) of Gram-negative bacteria. Its value for the functional and structural integrity of the outer membrane of a bacterium and the role that it plays in the endotoxicity of the LPSs are well known [1, 2]. In spite of great advances in the study of the structure and physiological activity of lipid A [3, 4] it continues to remain in the center of attention of research workers. Specialists in the field of molecular biology consider it an important probe in evaluating the mechanisms of cellular interaction [5, 6]. Chemists and biochemists use lipid A for studying the interrelationship between structure and function in biological processes [1, 7]. The capacity of lipid A for causing the polyclonal activation of B-lymphocytes [8, 9] provides immunologists with the possibility of understanding the mechanism of the poliferation and differentiation of B-cells, and its immunomodulating [10], antitumoral [11], and other properties [12] permit a hope for the use of lipid A or its derivatives as drugs in the not too distant future [13, 14].

The task of the present review is to generalize the voluminous literature information on the chemical and biological study of lipid A. In it, our main attention will be concentrated on investigations of recent years, since earlier reports have been fairly fully systematized in reviews published previously [1, 3, 4, 15-19]. In addition, it is just recently that great progress has been recorded in the investigation of the fine structure of lipid A [20, 21] and its biological properties [19].

CHEMICAL STRUCTURE OF LIPID A

The study of the structure of lipid A began fairly late, although the presence of a lipid component in endotoxins was known long ago. Attempts to isolate lipid A from an endotoxin or from a microbial cell by extraction with various solvents were unsuccessful, and it was concluded that lipid A is a fragment of an LPS molecule. Later [22] it was established that the lipid and carbohydrate components of the LPSs are linked with one another through a 2-keto-3-deoxy-D-manno-octonic acid (KDO) by means of a ketosidic bond. Lipid A is therefore obtained in the free state by mild acid hydrolysis [3, 5, 18, 19, 23-26]. The isolation of the lipid A is accompanied by its partial degradation as a consequence of the presence of acid-labile bonds in its molecule.

A characteristic feature of isolated lipid A is its heterogeneity due both to the natural imhomogeneity that is characteristic of it [27-33] and to degradation during hydrolysis [30]. Lipid A is usually a mixture of compounds that can be separated into fractions with the aid of successive extraction and fractional precipitation or chromatography [3, 29, 33]. The use of high-performance liquid chromatography enables individual compounds to be obtained [34].

Isolated lipid A is insoluble in acetone and methanol and is soluble in chloroform, pyridine, and water in the presence of triethylamine (TEA) [3, 15]. It melts with decomposition and has a negative optical rotation [3] and a molecular mass in the range of 1.7-2.0 kDa [3, 34, 35]. Samples of lipid A from different bacteria are close in composition and

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contain D-glucosamine (~20%), phosphorus (~2%), and fatty acids (~60%) [3, 15, 19]. The polyamines and metal cations present as counter-ions can be eliminated by high-voltage electrophoresis, ion-exchange chromatography, or electrodialysis, which considerably increases the solubility of the lipid A in water (in the form of the triethylammonium salt) [3]. Proteins or peptides are also frequently present in samples of lipid A, and according to some statements [36] they are bound to it covalently, while according to others [37] they form complexes.

Advances in establishing the structure of undegraded lipid A have become possible thanks to the selection of mutant strains of bacteria that synthesize incomplete LPSs deprived of carbohydrate chains. Such LPSs, so-called Re-glycolipids, contain only KDO and lipid A and form a convenient object for the structural study of the latter [27, 38, 39]. Thus, as early as 1969, from a Re mutant of Salmonella minnesota a phosphorylated pentasaccharide was isolated and characterized that had the following structure [38]:

$$\begin{array}{c} \mathrm{P\,.....\,GlcN} \xrightarrow{\beta\text{-}1,6} \mathrm{GlcN\,.....\,P} \\ \uparrow{\scriptstyle 2,3} \\ (\mathrm{KDO})_3 \end{array}$$

The β-1,6-glucosaminobiose forming a component of this pentasaccharide represents the carbohydrate skeleton of lipid A. It is obtained by a scheme proposed by Rietschel et al. [40]. This includes alkaline degradation of the LPS or of lipid A, reduction, hydrazinolysis, and N-acetylation. The structure of the carbohydrate skeleton modified in this way has been established by the usual methods and expedients of carbohydrate chemistry [3, 16, 19, 40-43].

Recently, in structural studies of biopolymers the method of nuclear magnetic resonance spectroscopy on carbon nuclei has found wide use. Its employment for the study of the structure of the carbohydrate skeleton of lipid A has definite advantages, since it provides the possibility of determining not only the type of glycosidic bond in the oligosaccharide but also its configuration [44, 45].

It must be pointed out that in addition to bacteria synthesizing lipid A with the "classical" carbohydrate skeleton [3, 15, 19, 40, 43-45], a group of microorganisms exists the lipid A of which contains an unusual carbohydrate skeleton. In one of them, in addition to β-1,6-glucosaminobiose, residues of other monosaccharides have been detected [46, 47], and in others the carbohydrate moiety of lipid A consists of residues of 2,3-diamino-2,3-dideoxy-D-glucose [17, 25, 48].

The lipid A molecule contains two phosphoric acid residues. Their positions in the molecule have been determined by using a combination of chemical [21, 37, 49] and physicochemical [28, 39, 50-53] methods. It has been established that one phosphate group is present at the C-1 atom of the reducing and the other at C-4' of the nonreducing disaccharide residue. The phosphate groups of the lipid A of many microorganisms are substituted by polar groups. Thus, in lipid A from Chromobacterium violaceum [54] a D-glucosamine residue is attached to the phosphate at C-1, and the second phosphate group is substituted by 4-amino-4-deoxy-L-arabinose:

$$L\text{-Ara4N}\xrightarrow{1}\mathrm{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}\xrightarrow{1}\mathrm{P}\xleftarrow{1}\mathrm{GlcN}.$$

The nature of the substituents, their number, and the order of their distribution in lipid A from different bacteria may vary:

Escherichia coli [27, 28, 39]

$$\mathrm{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\mathrm{P}-\mathrm{P};$$

Rhodospirillum tenue [46]

$$\begin{array}{l} L\text{-Ara4N}-\mathrm{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\mathrm{P}-\mathrm{Ara}_f; \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\uparrow{\scriptstyle 1,4} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\mathrm{GlcN} \end{array}$$

Yersinia pestis [53]

$$L\text{-Ara4N}-\mathrm{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\mathrm{P}-\mathrm{Ara}_f;$$

Proteus mirabilis [35]

$$L\text{-Ara4N}-\text{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\text{P}.$$

In the Re-glycolipid from S. minnesota [19], the carbohydrate skeleton consists of a trisaccharide having the following structure:

$$L\text{-Ara4N}-\text{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\text{P}-\text{P}.$$

It is interesting that the precursor of lipid A isolated from bacteria of the same species has a different order of distribution of the substituents [51]:

$$\text{P}-\text{P}-D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}D\text{-GlcN}-\text{P}-L\text{-Ara4N}.$$

The monosaccharide residues are attached to the phosphate groups by glycosidic bonds and are not acylated. Their presence in the molecule is of great functional significance. It has been shown, for example, that LPSs the lipid A of which contains aminoarabinose are more resistant to the action of polymyxin than those in the composition of which this aminosugar has not been detected [55].

A hypothesis has been expressed previously that lipid A has an oligomeric structure consisting of repeating disaccharide subunits linked with one another by phosphodiester or pyrophosphate bridges [15]. In actual fact, pyrophosphate groups have been detected in lipid A and LPSs, but careful structural studies have shown that they do not participate in the formation of the bond between the subunits.

Until recently, it was considered that phosphorus was an obligatory component of lipid A. Later, LPSs became known the lipid A of which contains no phosphate group [47, 48, 56] – for example, that of Rhodomicrobium vannielii [47]:

$$\text{Man}\xrightarrow{1,1}D\text{-GlcN}\xrightarrow{\beta\text{-}1,6}\text{GlcN}$$

An important structural element of lipid A, determining its hydrophobicity, consists of fatty acids. Among the acids present in lipid A the main ones that have been detected are the normal and 3-hydroxy carboxylic acids with long carbon chains. As a rule, the hydroxy acids predominate, making up 50-75% of the total amount of acids [3, 15]. With rare exceptions [8, 47, 49], unsaturated fatty acids are not found in lipid A. The α,β- and β,γ-unsaturated acids detected in hydrolysates of lipid A are products of the degradation of the hydroxy acids during hydrolysis [57].

The fatty acid residues are attached to the carbohydrate skeleton by ester and amide bonds. Among the acids acylating the hydroxy groups of D-glucosamine, normal acids and 3-hydroxy acids with even numbers of carbon atoms predominate. According to more modern ideas, the normal fatty acids are not bound directly to the glucosamine but acylate the hydroxy groups of the hydroxy acids [3, 15, 57, 58]. In the lipid A from a number of bacteria of the family Rhodospirillaceae the hydroxy groups of the carbon skeleton are not esterified [48].

The amino groups of the glucosamine residues are acylated by 3-hydroxy acids. 3-Hydroxytetradecanoic acid [3, 15] and its O-acyl derivatives are found most frequently [59-61]; however, other 3-hydroxyalkanoic acids are also found [3]. Among the acids acylating hydroxy groups in hydroxy acids, dodecanoic, tetradecanoic, and other normal fatty acids have been detected [59-61]. In the lipids A of some bacteria of the family Rhodospirillaceae one of the amino groups of the disaccharide is substituted by a 3-oxotetradecanoic residue [49, 56]. All the 3-hydroxy acids investigated have the R(–) configuration, while the 2-hydroxy acids present in the lipids A of a number of bacteria [3] have the S(+) configuration [57, 62].

Since 3-hydroxy acids are obligatory components of lipid A and, with rare exceptions [63], are not present in other bacterial lipids, they are used to detect endotoxins in serum [64], microbial mass [65], the soil [66], and other biological materials [67].

The composition of the fatty acids is usually constant for a given lipid A and does not depend on the conditions of cultivation of the microorganism. Reports have recently appeared from which it follows that the growth of bacteria at low temperatures leads to a change in the fatty acid composition of the lipid A with an increase in the degree of its unsaturation [30, 68, 69].

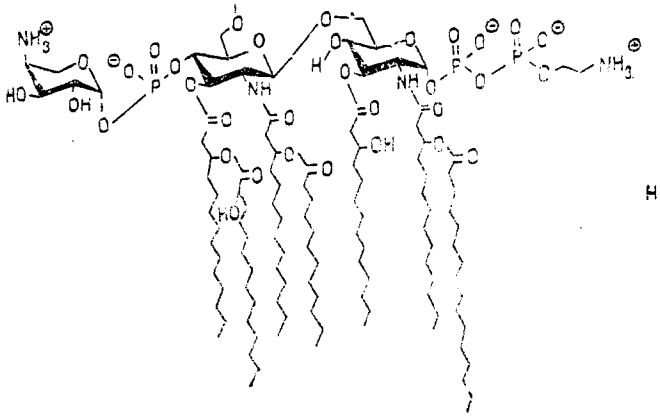

Fig. 1

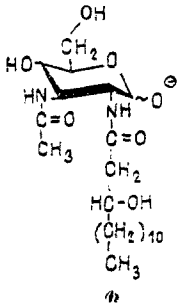

Fig. 2

Fig. 1. Structure of lipid A from Salmonella minnesota [76].

Fig. 2. Structure of lipid A from Rhodopseudomonas viridis [25].

Since the composition of the fatty acids of lipid A is constant within certain limits for members of a given genus or family, they can be used in the chemotaxonomy of bacteria [70].

Recent years have been marked by great advances in the establishment of the fine structure of lipid A. These became possible thanks to the use of nondestructive methods of analysis such as NMR spectroscopy [50, 53, 71-73], fast-atom mass spectrometry [34, 74], and laser mass spectrometry [35, 61, 75], which permit information to be obtained on the structure of a compound without its preliminary degradation. With their aid, the degree of acylation of the carbohydrate skeleton of lipid A by fatty acids has been determined, the distribution of these acids in the molecule of the glucosaminobiose has been studied, and the idea of the position of the bond of lipid A with the polysaccharide has been reconsidered. In the cases of Proteus mirabilis [35], S. minnesota [76], S. typhimurium [73], E. coli [77], Yersinia pestis [53], Rhodopseudomonas sphaeroides [49], and Rhodomicrobium vannielii [47], the information obtained has permitted an approach to the establishment of the complete structures of the lipids A.

The majority of samples of lipid A that have been characterized at the present time are constructed on a single principle. A typical representative of this type of molecule is the lipid A of S. minnesota (Fig. 1) [76].

The lipid A of this group of bacteria contains β-1,6-glucosaminobiose with phosphate groups at the C-1 and C-4' atoms. The phosphate groups may be substituted or remain free. The reducing end of lipid A has the α-configuration, and the bond with KDO is effected through the C-6' atom of GlcNII, the hydroxy group at the C-4 of GlcNI being unsubstituted. The amino groups are acylated by 3-hydroxy acid residues which, in their turn, may be acylated by fatty acid residues. The hydroxy groups at the C-3 and C-3' atoms are acylated by 3-hydroxy and 3-acyloxy acids. The differences in the structures of lipids A of this type from different bacteria are determined by the presence or absence of polar substituents, their nature and composition, and the type of distribution of the fatty acid residues.

A special position is occupied by the lipid A of a number of bacteria of the family Rhodospirillaceae. They consist of residues of 2,3-diamino-2,3-dideoxy-D-glucose acylated in the amino groups with 3-hydroxy acids and contain no phosphorus and no fatty acids with the ester type of bond [25] (Fig. 2).

Thus, the most frequently encountered type of lipid A consists of a glycophospholipid with a unique structure based on a polyhydroxy compound that is unusual for this class of substances – glucosaminobiose acylated by rare 3-hydroxy and 3-acyloxy acids that are encountered only in endotoxins. The presence in the molecule of hydrophilic (phosphorylated glucosaminobiose) and hydrophobic (fatty acid) sections makes lipid A an amphipathic compound. Furthermore, it is amphoteric, since it contains acid (phosphate) and basic (L-AraN and polyamine) groups. According to the results of x-ray structural analysis, the fatty acids of lipid A are oriented in one direction and are present in hexagonal dense packing

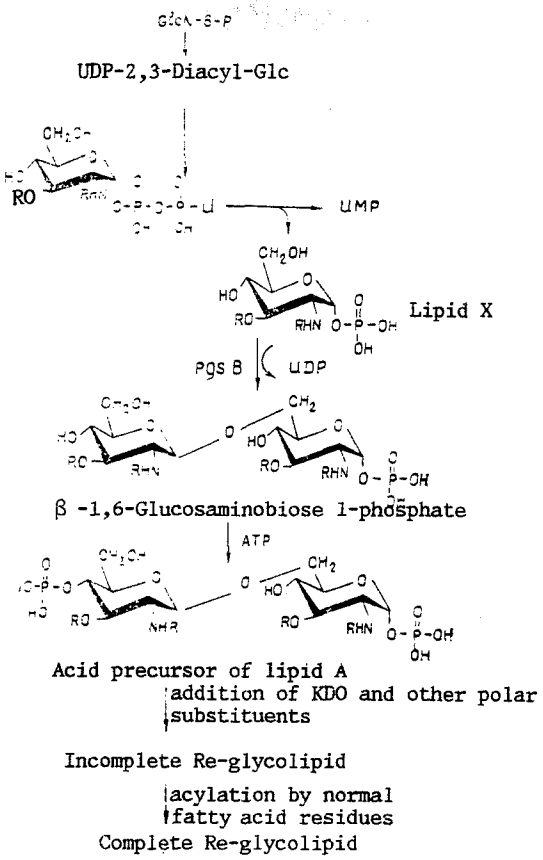

Fig. 3. Hypothetical scheme of the biosynthesis of lipid A [93-95] (R represents a 3-hydroxytetradecanoic acid residue).

[20, 78-80]. The highly ordered state of the hydrophobic moiety, which is favored by the absence of unsaturated fatty acids, makes the structure of lipid A relatively rigid, in comparison with other lipids, and is an important condition for the manifestation of its properties in the membrane. It is assumed [20] that the barrier functions of the outer membrane of Gram-negative bacteria, limiting the entry of hydrophobic substances, are ensured by the comparatively rigid and well-ordered conformation of lipid A. This appears particularly important from the therapeutic point of view since it is known that to detoxify Gram-negative bacteria higher doses of antibiotics are frequently necessary than those used in the case of Gram-positive bacteria.

BIOSYNTHESIS OF LIPID A

Until recently, there was practically no information on the biosynthesis of lipid A. Prospects in this field of investigations were observed when a possibility appeared of obtaining mutant strains of bacteria [81, 82]. For so far unknown reasons, phosphorylated mono- [83-85] and disaccharide [86, 87] derivatives of glucosamine that are biosynthetic precursors of lipid A accumulated in bacteria with a disturbed synthesis of phosphatidylglycerol or of KDO. A detailed study of the structures of these compounds [84-89] and their use as acceptors in experiments on the assembly of certain elements of the outer membrane of Gram-negative bacteria [90-92] has permitted a hypothetical scheme of the biosynthesis of lipid A to be suggested (Fig. 3) [93-95].

According to modern ideas, the biosynthesis of lipid A takes place in two stages. First there is the synthesis of a monosaccharide precursor, so-called lipid X, consisting, in the structural respect, of the reducing end of lipid A (a 2,3-diacylglucosamine α-1-phosphate). In vitro experiments have demonstrated that lipid X can be obtained by the acylation of UDP-N-acetyl-D-glucosamine [96]. The latter, together with lipid X, participates in the formation of the disaccharide precursor of lipid A [92].

In the second stage, a phosphate group is introduced into the 4'-position of the glucosaminobiose. This forms an acid precursor [93, 94] which is the key compound in the bio-

synthesis of lipid A and acts as the common acceptor of all the polar substituents: KDO, L-aminoarabinose, and phosphorylethanolamine [95]. In the last stage of the synthesis of lipid A saturated fatty acid residues acylating the hydroxy groups of the hydroxy acids are introduced into its molecule.

The proposed scheme of biosynthesis cannot be regarded as definitive. With the development of methods of enzymological investigations, new facts are becoming available which supplement the pattern of biosynthesis of lipid A and reveal the role of the latter in the assembly of the outer membrane of Gram-negative bacteria. They permit an understanding of the mechanisms controlling the process of biosynthesis and facilitate the isolation of fragments of DNA coding the synthesis of the enzymes. An understanding of these phenomena makes it possible to create an effective in vitro system for obtaining lipid A analogues of given structure.

BIOLOGICAL PROPERTIES OF LIPID A

It is well known [4] that the LPSs of Gram-negative bacteria act on practically all systems of the organisms, causing serious pathophysiological changes. Many of these resemble processes observed in patients with Gram-negative bacteremia and sepsis [97]. The possible role of lipid A in the appearance of these reactions has been studied in various laboratories [1-4, 15-18], and it has been shown that it is just this substance that is the active center of the endotoxins. The water-insolubility of lipid A, its heterogeneity and its degradation during isolation have considerably complicated the study of its physiological activity. Progress in this field of investigations was recorded when the possibility appeared of working with soluble preparations, in which Re-glycolipids or complexes of lipid A with proteins [15], TEA [98], or liposomes [10] were used. The great advances achieved in recent years in obtaining biosynthetic precursors of lipid A [83, 84, 86-88] and its synthetic analogues with diverse structures [99-101], which, in contrast to a native sample, have been well characterized chemically, have, finally, permitted a close approach to answering the complex question of the interrelationship of the structure and function of lipid A.

The whole complex of physiological activity of lipid A can be divided into three groups: endotoxic properties; stimulation of the nonspecific resistance of the macroorganism; and immunomodulating properties.

ENDOTOXIC PROPERTIES OF LIPID A

The classical manifestation of the endotoxic properties of lipid A is toxicity [3, 4, 15, 18]. In small doses it causes the death of the animal, including chick embryos. Sensitivity to it increases in the presence of a number of substances such as lead acetate [102], some drugs [103], and D-galactosamine [104].

It is considered that hydroxy acids with the ester type of bond play an important role in the manifestation of toxicity. Samples of lipid A in which the hydroxy groups of the carbohydrate skeleton are not acylated or contain, in place of 3-hydroxytetradecanoic acid, the corresponding 3-oxo acid are not toxic [105, 106]; at least one of the hydroxy acids with the ester type of bond must be acylated by the residue of a normal fatty acid [107]. The presence of two acyloxy acids leads to a fall in toxicity [108].

Apparently, the distribution of the fatty acid residues in the glucosaminobiose molecule has a fundamental influence on the toxicity of lipid A. A completely synthetic analogue of lipid A with a fatty acid residue at the C-6' atom was weakly toxic [109], while an analogous compound acylated not at the C-6' but at the C-3' atom had a toxicity comparable with that of *E. coli* lipid A [110]. On the other hand, lipid A having a high degree of acylation but with no phosphate at the C-1 atom was not toxic [34]. Samples of lipid A containing no phosphorus whatever were not toxic, either [48].

The intravenous injection of lipid A leads to a rise in the body temperature of experimental animals [3, 4, 18]. The pyrogenic effect has a two-phase nature and reaches maxima after one and three hours [3]. The formation of a complex of lipid A with a protein carrier enhances the effect, and an influence of the nature of the carrier on the pyrogenic activity of the complex has been observed [3]. Samples of lipid A not containing phosphorus [48] or O-acyl substituents [105, 106] are weakly pyrogenic. Even the partial deacetylation of lipid A increases its pyrogenicity [3, 4]. The height of the pyrogenic response of animals depends directly on the nature of the substituent at the C-3 atom of the glucosaminobiose [111].

Compounds having no substituents in this position are practically inactive; the introduction of a 3-hydroxy acid residue considerably raises the pyrogenicity of the sample; the replacement of the 3-hydroxy acid by a 3-acyloxy derivative leads to compounds with a high pyrogenic response. The monophosphate of lipid A is practically inactive in this test, which shows the importance of the presence of a phosphate group at the C-1 atom for the existence of pyrogenic activity [107].

A characteristic endotoxic property of lipid A is its capacity for causing a local Shwartzmann reaction in experimental animals [3, 4, 14, 18]. A cutaneous Shwartzmann reaction is considered to be an in vivo model of disturbances in the blood-clotting system caused by an endotoxin [4]. Decisive importance in the manifestation of this property is possessed by the acyloxy acids; their elimination leads to compounds inactive in the Shwartzmann reaction. Synthetic analogues of lipid A and also its precursors isolated from mutant strains of bacteria not having the above-mentioned fatty acid residues are incapable of causing tissue necrosis [111, 112]. On the other hand, the elimination of even one phosphate group likewise makes lipid A inactive [107].

Among the important properties of lipid A is its capacity for causing the gelation of a lysate of amebocytes of the king crab Limulus polyphemus, so-called LAL activity [3]. This reaction is sensitive and specific, and is widely used to detect endotoxins [113, 114]. The inclusion of free lipid A in liposomes lowers its gelating action by a factor of 10^5. This demonstrates the participation of the hydrophobic sections of the molecule in the manifestation of LAL activity. According to some results [115, 116], glucosamine acylated in the amino group with 3-hydroxytetradecanoic acid possesses a weak gelating action. According to others [117], the presence of at least one phosphate group is necessary.

STIMULATION OF THE NONSPECIFIC RESISTANCE OF THE MACROORGANISM

The administration of lipid A raises the general resistance of the animal organism. The activating action of lipid A on the complement system is particularly significant [3, 4, 14, 18]. The activation of this complement system by lipid A takes place by the classical pathway [118] and depends on the degree of aggregation of the lipid molecules [119]. The chemical modification of lipid A leading to a loss of toxicity by it gives rise to a simultaneous considerable fall in anticomplement activity [120, 121]. However, the latter is not connected with toxicity, since there are nontoxic samples of lipid A which possess anticomplementary activity [18].

One of the important factors of the nonspecific stimulation of the macroorganism by lipid A is the activation by it of the phagocytosis system, leading to an enhanced secretion of prostaglandin, interleukin, and interferon by the macrophages [122]. The study of the structural features necessary for the production of interferon has revealed no definite functional groups [123, 124]. The maximum response is observed only when all the structural elements are present [111]. Conversely, the synthesis of interleukin and of prostaglandin E_2 was observed on the administration to animals of compounds of fairly simple structure corresponding to the reducing end of lipid A [125].

It is assumed that before lipid A becomes capable of causing the stimulation of the cells of the macroorganism (lymphocytes, macrophages) it must be somewhat modified (deacylated) and must not contain nonhydroxy acids [126]. It has been shown that human polymorphonuclear leukocytes and murine macrophages contain enzymes capable of deacylating and dephosphorylating lipid A or LPSs [127, 128].

In vitro and in vivo experiments show that lipid A can be used in the treatment of malignant tumors [11, 13]. It stimulates the induction of tumor necrosis factor by the macrophages [129] and synergistically enhances the capacity of adjuvants from the cell walls of mycobacteria for causing the regression of solid tumors [130]. Here rapid and complete disengagement of the tumor, with the elimination of metastases in the peripheral lymph nodes, and systematic immunity to a transplanted tumor are observed. Ribi et al. [11, 15, 36] have succeeded in obtaining a nontoxic derivative of lipid A in the form of its 4'-monophosphate with an antitumoral action comparable with the action of the initial glycolipid. At the present time, attempts are being undertaken to use this preparation for the treatment of malignant tumors of man [13].

IMMUNOMODULATING PROPERTIES OF LIPID A

Lipid A belongs to the concealed antigens and its antigenic properties appear only when it is present in the isolated state [131]. The immunogenicity of free lipid A is weak [10, 132] but it rises considerably when lipid A is complexed with suitable carriers: proteins [133], liposomes [10, 31], erythrocytes [134], or partially hydrolyzed homologous bacteria [3, 18, 135]. The immunization of experimental animals with such complexes leads to the formation of antibodies that interact with the free lipid A or its de-O-acylated derivatives but do not react either with LPSs or with bacterial cells [131].

Samples of lipid A from different bacteria give cross-serological reactions [3, 18, 134], on the basis of which a common antigenic determinant has been deduced. Various groups of workers [120, 132, 134-136], using natural and synthetic samples of lipid A, have shown that the compound of simplest structure that exhibits the antigenic specificity of lipid A is D-glucosamine phosphate acylated with a 3-hydroxy acid residue. The nature of the fatty acid acylating the amino group is not of fundamental significance [132, 135], and the phosphate groups play the part of a solubilizing agent [3]. Further investigations demonstrated that phosphate groups and fatty acids with ester bonds participate in the formation of the amino determinant group of lipid A [137, 138]. According to other authors, the latter are not components of the immunodeterminant of lipid A [139].

Antibodies to lipid A are present in the normal sera of many species of animals and of man [3, 15, 140]. Their level rises when a Gram-negative infection is present, for example, in patients with bacteremia and sepsis [141, 142] and with chronic infection of the urogenital tract [141, 143], and this can be used for the diagnosis of these diseases [144, 145]. A possible explanation of the raised level of antibodies to lipid A may be the constant presence of the latter in kidney tissue. As has been shown [146], lipid A is detected in the kidneys for six weeks after its injection into the animal organism. On the other hand, a lowered level of antibodies to lipid A in patients with monoclonal B-cell malignancies [147] is accompanied by an increased sensitivity to a Gram-negative infection and indirectly witnesses the protective role of the antibodies to lipid A.

The possible role of antibodies to lipid A, the common structural component of endotoxins, in protection from bacterial infection or the toxic action of LPSs has long attracted the attention of scientists [114, 148]. Thus, it has been shown that antibodies to lipid A opsonize E. coli bacteria for subsequent intraperitoneal phagocytosis in mice [134, 139] and protect the mice from experimental salmonella infection [134]. In rabbits, under definite conditions, antibodies to lipid A suppress the pyrogenic activity of LPSs and of lipid A and inhibit the local Shwartzmann reaction [150]. At the same time, there have been other reports in which the protective action of antibodies to lipid A has not been confirmed [151]. Nevertheless, attempts are known to use human sera with high levels of antibodies to lipid A in clinical medicine for the treatment of sepsis patients [14].

Recently, the attention of research workers has been attracted to the possibility of using for protection against bacterial infection antibodies to the core section of endotoxins [152]. They have a high degree of cross-reactivity with a large range of Gram-negative bacteria. The study of the specificity of these antibodies has shown that the antigenic determinants, in response to which the synthesis of antibodies possessing protective properties begins, are localized in the lipid section of the LPS [152]. A report on the neutralization of the lethal action of endotoxins in mice with the aid of a compound of such a simple structure as lipid X is considered encouraging [153].

The presence of lipid A can considerably enhance the immune response to weak antigens. A study of the adjuvant properties of lipid A and its synthetic analogues [154-156] has shown the importance of a phosphate group at the C-1 atom of the disaccharide fragment. The presence of a second phosphate group is not obligatory. The nature of the acid acylating the amino group of the glucosamine has no fundamental influence on the adjuvant activity. The partial deacylation of lipid A leads to a considerable fall in the adjuvant action of the compounds obtained [155].

It is assumed that a direct link exists between the adjuvant effect and the mitogenic action of lipid A [157]. In small doses, lipid A initiated the proliferation of B-lymphocytes [3, 5, 6] while the mitogenic action falls strongly if the lipid A is de-O-acylated. The importance of the substituent at the C-3' atom of the glucosamine has been shown: The selective elimination of a hydroxy acid residue from this position leads to the complete loss of

mitogenicity, while the replacement of the hydroxy acid residue by a 3-acyloxy acid residue enhances the mitogenic effect of the corresponding derivative. The presumed mechanism of the triggering of the proliferation of the B-cells is that lipid A interacts with the cell membrane of the B-lymphocytes or is introduced into it, being bound with membrane receptors (or enzymes) through the section of the bond of the 3-hydroxy acid with the hydroxy group at the C-3 atom of glucosamine [158].

Until recently, it was considered that the mitogenic action of lipid A was limited to B-lymphocytes. However, there are results [159] showing that lipid A exhibits a mitogenic effect on the population of T-cells and promotes their cooperation with the B-lymphocytes in the process of forming the humoral response.

Polyclonal activation of the B-lymphocytes is observed under the action of lipid A [8, 9]. Macrophages apparently participate in this process since the elimination of the latter from a suspension of cells considerably lowered the action of lipid A as a polyclonal activator [9].

Generalizing the information given in this section of the review, it is possible to single out a number of structural features important from the point of view of the biological activity of lipid A. Thus, the presence of 3-hydroxy acid residues in the amino groups of the glucosaminobiose appears obligatory, since they apparently implement the triggering mechanism. The phosphate group at the C-1 atom of the glucosaminobiose must be regarded as an important functional group. Its elimination leads to a considerable fall in the endotoxic reactions of lipid A, in its capacity for interacting with the immune system of the macroorganism, and in a number of other properties. The introduction of a 4-phosphate group enhances the manifestation of certain properties, particularly those that relate to the immunomodulating activity. An analogous action is exerted by 3-hydroxy acids with the ester-type bonds and their acyloxy derivatives. The nature of the influence of various structural elements on the activity of lipid A depends on the balance of the hydrophobicity and hydrophilicity of the molecule as a whole.

Thus, lipid A possesses a broad spectrum of physiological activity and forms a common covalently bound lipid component of the LPSs of Gram-negative bacteria. Thanks to the collective forces of numerous investigators the structure of this unique compound has been elucidated, and the functional groups responsible for the manifestation of various biological properties have been determined. At the present time, the interest of research workers is shifting in the direction of the use of the information obtained in the practical activity of man. The possibility of obtaining nontoxic samples of lipid A retaining their useful properties must be specially mentioned [160-163].

The great advances achieved in the field of the synthesis of lipid A analogues and the prospects noted in the study of the biosynthesis of the endotoxin permit the hope that in the very near future highly active immunomodulating substances will be obtained possessing antitumoral and antiendotoxic activity; the principles of the biogenesis of bacterial membranes and the mechanisms upon which the differentiation of animal cells are based are becoming known.

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