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MINIREVIEW

Lipopolysaccharide Nomenclature—Past, Present, and Future†

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Since their discovery about 100 years ago, bacterial endotoxins have been among the most widely studied of all bacterial components. The interest in endotoxin is based, in large part, on the diversity of biological responses which this molecule elicits both in vitro and in vivo. A second major reason for this interest is that the biologically active moiety of endotoxin, the lipopolysaccharide (LPS), has been identified and biochemically characterized for a variety of enteric organisms. Further, the toxic principle of LPS, lipid A, has been defined and synthesized. Thus, it appears that many of the mysteries still surrounding the mode of action of this unique bacterial constituent will soon be resolved at the molecular level.

Most reports on LPSs deal with those from enteric organisms. Recently there has been renewed interest in the LPS of gram-negative pathogens and in the LPS of nonenteric mucosal pathogens in particular. As we mark the beginning of a new chapter in the study of these biologically active molecules, it seems appropriate to review the nomenclature of LPS, with special emphasis on the applicability of current terms to the LPS found in nonenteric pathogens.

Historical perspective. The endotoxin story began with empirical observations of fever as a clinical sign of disease; these date back to the beginning of recorded medical history (reviewed in references 53 and 57). Two philosophies evolved concerning the significance of fever, one holding that it is a deleterious consequence of disease and the other holding that it is a defense mechanism against disease. Investigations from the second viewpoint led to the discovery, in putrefying tissues, of materials called pyrogens which, when injected intravenously into experimental animals, induced fever. From these observations developed the concept of a pyrogenic principal. Over a century later, it was shown that trichloroacetic acid extracts of gram-negative bacteria could induce fever. The pyrogenic activity of these extracts was postulated to be caused by endotoxins, a possibility first proposed by Richard Pfeiffer 100 years ago (cited in reference 57). The term endotoxin became widely used to denote the toxic principle of gram-negative bacteria; systemic exposure to endotoxin in humans or other mammals results in numerous adverse sequelae, in addition to fever, which are collectively termed "endotoxicity" (reviewed in reference 6). First isolated and described by Boivin and Messrobeanu (1), Morgan (34), and Goebel et al. (8), endotoxin consists of an outer membrane macromolecular complex of polysaccharide, lipid, and protein. Two decades after these findings, Westphal and co-workers (55) reported the successful extraction and purification of protein-free LPS. LPS, a molecule of covalently bound lipid and carbohydrate (reviewed in references 6, 27, 29, 42, 44, 54), was separated from the bacterial cell wall and from protein and other constituents by extraction with hot aqueous phenol; LPS possessed all of the biological activity of endotoxin (55). Subsequently, the lipid portion (lipid A) of the LPS molecule was isolated, characterized, and shown to be the minimum structure necessary for pyrogenicity and endotoxicity (55). Most recently, lipid A has been organically synthesized; this synthetic lipid A molecule possesses the same biological properties as bacterial free lipid A (5, 22, 23, 48, 49).

Basic structure of LPS. LPS from members of the family *Enterobacteriaceae* has been structurally analyzed (Fig. 1). Three well-defined regions have been described: (i) the O-specific antigen, also called O-antigen or somatic antigen, which is a long-chain polysaccharide consisting of repeating units containing one to seven sugars, i.e., monosaccharides (reviewed in references 19, 28, 42); (ii) the oligosaccharide core, composed of approximately 10 monosaccharides (including 3-keto-2-deoxyoctonate [KDO], heptose, glucose, galactose, and *N'*-acetylglucosamine) which are added sequentially by unique glycosyl transferases; and (iii) lipid A, a unique lipid backbone consisting of diglucosamine in β1-6 linkage with ester-, amide-, and diester-linked fatty acids (the type and position of fatty acids vary among species) and with phosphate, 4-amino-arabinose substituents, or both on the reducing and nonreducing sugars. Mild-acid hydrolysis techniques cleave lipid A from the core and O-antigen polysaccharide generating free lipid A and degraded polysaccharide. In soluble form (electrodialyzed salt form), free lipid A possesses most of the endotoxic and biological characteristics of intact LPS (reviewed in references 6, 44, 45).

Smooth versus rough LPS. The LPSs that were first isolated from enteric bacteria and characterized were structurally similar; all contained O-antigen (Fig. 2). The long surface-exposed polysaccharide constituting the O-antigen confers two characteristics on these bacteria: (i) the edges of the bacterial colonies are smooth and entire, and (ii) the bacteria do not autoagglutinate in saline. LPS mutants have been isolated which lack O-antigen. These bacteria differ from the wild-type organisms in two ways: (i) the edges of the bacterial colonies are irregular and rough, and (ii) the

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† Dedicated to the memory of our coauthor Dr. Loretta Leive, who died on February 12, 1986, in Washington D.C.

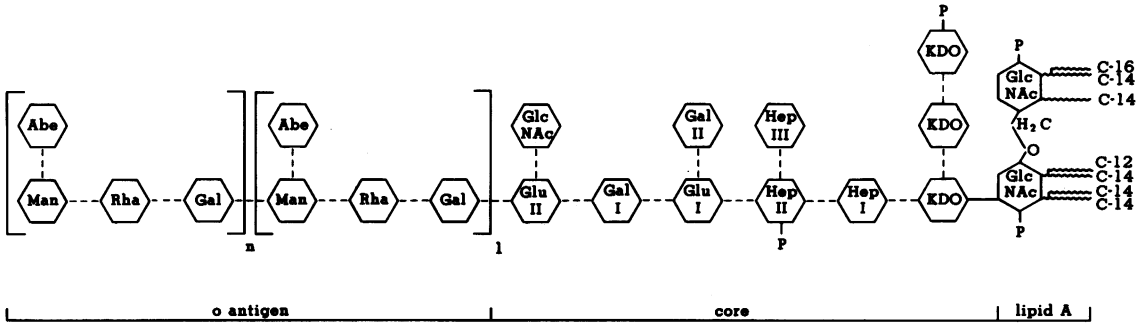

FIG. 1. Schematic diagram of the smooth LPS of *S. typhimurium*. The lipid backbone (lipid A) is composed of β1-6-linked *N*-acetylated glucosamine monosaccharides (GlcNAc). The seven fatty acids (~~~) are C-12, C-14, C-16, OH-C-16, and OH-C-12. These are present in either amino or hydroxyl linkage. (The number and type of lipid A fatty acids vary among bacterial species.) In addition, phosphate esters (P), amino groups (not shown), or both are substituted at the 1 and 4′ positions of the nonreducing and reducing glucosamines, respectively. An eight-carbon keto sugar, KDO, links (6′) the lipid A and polysaccharide portions of the molecule; the number of KDOs is also variable among species. The remainder of the core sugars include heptose (Hep), glucose (Glu), galactose (Gal), and *N*-acetylglucosamine (GlcNAc). Some of the core sugars may be modified by both phosphate and amino groups. These are not necessarily present in stoichiometric amounts. The O-antigen sugars in *S. typhimurium* include galactose, rhamnose (Rha), mannose (Man), and abequose (Abe). In addition, galactose may be modified by glucose in approximately 30% of the LPS molecules (30, 44).

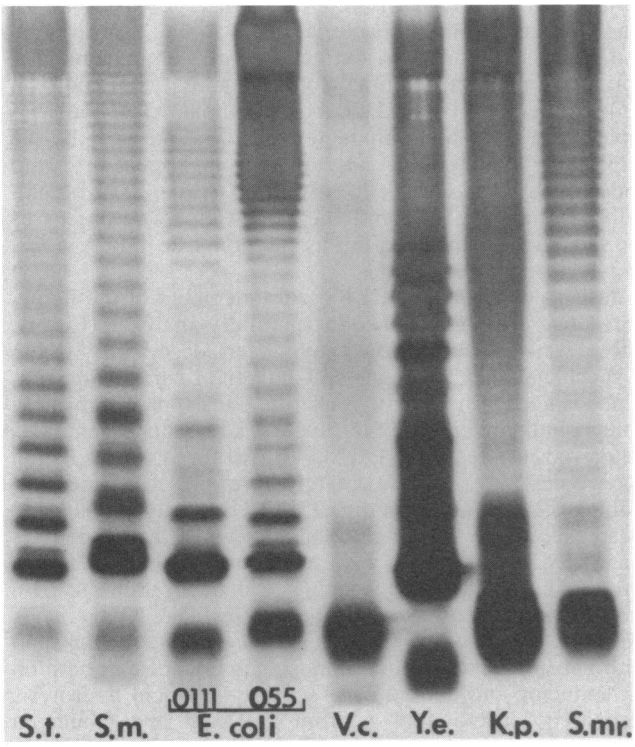

FIG. 2. Silver-stained sodium dodecyl sulfate-polyacrylamide gel (12.5%) containing 2 μg of smooth LPS per lane (11). S.t., *S. typhimurium*; S.m., *S. minnesota*; 0111 and 055, *E. coli* strains; V.c., *Vibrio cholerae*; Y.e., *Yersinia enterocolitica*; K.p., *Klebsiella pneumonia*; S.mr., *Serratia marsescens*. Note the doublet ladder characteristic of most enteric LPSs (except *V. cholerae*) which is thought to represent LPS molecules with increasing numbers of O-antigen units (9, 18, 43). The reason for the doublet banding pattern is unknown. It has been postulated that the variation in phosphorylation might explain this difference in M_r; however, there is no experimental evidence to support this hypothesis (9). In addition to a slight difference in M_r, the bands of the doublet silver stain differently (upper, grey; lower, red) (11). *S. typhimurium* and *S. minnesota* LPSs were extracted by the method of Westphal et al. (55). The other preparations were purchased from List Biological Labs, Inc., Campbell, Calif.

bacteria autoagglutinate in saline. Because of these differences in colonial morphology and autoagglutination, LPSs which contain O-antigen are denoted as smooth (S) and those which lack O-antigen are denoted as rough (R) (46). "Wild type" is used to indicate the naturally occurring phenotype of the organism; for the LPSs of the enterics, "wild-type LPS" has been used to denote smooth LPS. However, that connotation is not inherent in the term.

Understanding of the LPS biosynthetic pathways and of the structure of the LPS molecule has been greatly facilitated

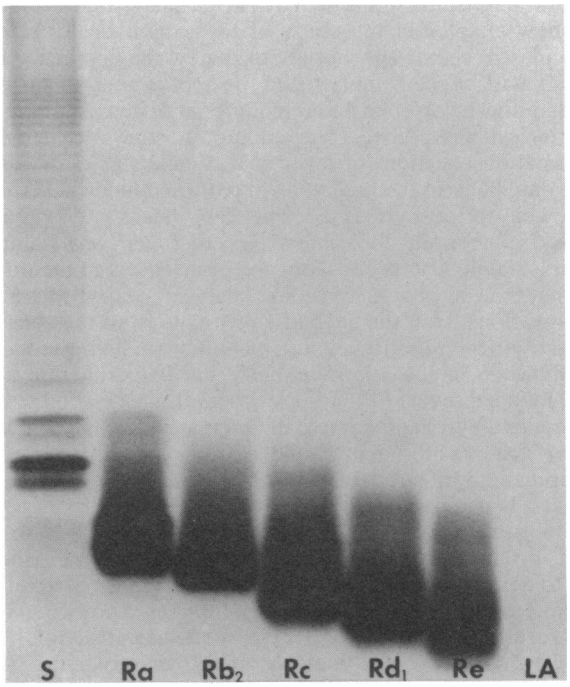

FIG. 3. Silver-stained sodium dodecyl sulfate-polyacrylamide gel (18%) containing 2 μg of *S. minnesota* smooth (S) and rough (Ra, Rb_2, Rc, Rd_1, Re) chemotypes and free lipid A (LA). Lipid A does not stain by this method (11). The rough LPSs lack the doublet ladder associated with smooth LPSs (11, 36). Rough LPSs were purchased from List Biological Labs, Inc.

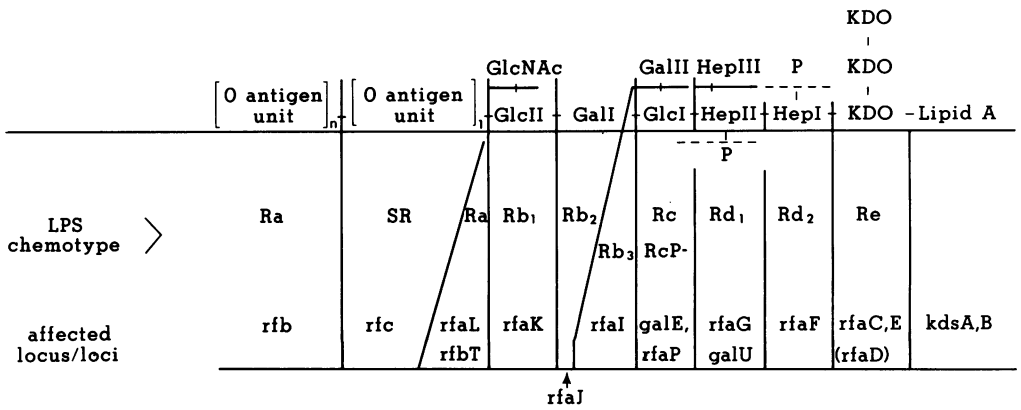

FIG. 4. Schematic diagram of the *S. typhimurium* LPS. The locus or loci of affected genes for each LPS chemotype are identified. These loci are described in Table 1. Adapted from reference 30.

by the selection and analyses of a number of specific LPS mutants of *Salmonella minnesota* and *Salmonella typhimurium*. These organisms have defects in genes which code for glycosyl or phosphoryl transferases (or epimerases); as a result, a truncated LPS molecule is synthesized, since the omission of any sugar in the core will interfere with the addition of distal sugars including the O-antigen (Fig. 3). These mutations occur primarily in two gene clusters, which have been named rough A (*rfa*) and rough B (*rfb*); the mutants have been designated rough, R, or core mutants. All core mutant LPS lacks covalently bound O-antigen. The length of the oligosaccharide core ranges from that of the Re to that of the Ra chemotypes; the term chemotype is used synonymously with the term LPS phenotype. Re LPS is composed of lipid A and two to three molecules of an eight-carbon sugar, KDO. (Re LPS is sometimes referred to as deep rough or heptoseless LPS.) It appears that Re LPS is the minimum requirement to maintain the integrity of the gram-negative outer membrane. Only temperature-sensitive mutants with genetic defects in KDO synthesis have been isolated; when the organisms are shifted to permissive temperatures, the cells stop growing and die. The Ra chemotype is composed of lipid A and a complete core but lacks covalently bound O-antigen. The Ra mutant is a manifestation of a genetic defect in the *rfaL* or *rfbT* gene; defects in other *rfb* genes may also result in synthesis of the Ra chemotype. One additional mutant should be mentioned, although it is not a true core mutant since the resulting LPS phenotype is not rough. A defect in a gene in a third cluster, rough C (*rfc*), results in the absence of O-antigen polymerase activity. The resulting semirough (SR) LPS has lipid A, a complete core, and one covalently bound O-antigen unit. These and other LPS chemotypes and genetic defects are summarized in Fig. 4 and in Table 1 and are reviewed in great detail in reference 30.

Although it is possible to characterize LPS mutants by their core and O-antigen structures, it is nevertheless important to recognize that, in the case of wild-type organisms which have predominantly smooth LPS, there is considerable size heterogeneity of the individual LPS molecules which collectively define the LPS of the outer membrane (9, 11, 18, 36, 43). In addition to the O-antigen-containing

TABLE 1. *S. typhimurium* locus or loci of affected genes for each LPS chemotype

Affected locus or loci	Phenotype	Presumed defect[a]	Synthesis of O-antigen	
			As hapten	Covalently bound to core
kdsA(Ts)	Lethal at permissive temp	KDO-8-P synthetase		
kdsB(Ts)	Lethal at permissive temp	KDO-CMP synthetase		
rfaE	Re	Heptosyl I transferase (?)	+	−
rfaC	Re	Heptosyl I transferase or biosynthesis of heptosyl donor	+	−
rfaD	Re and Rd_2	D-D–D-L Heptose epimerase	+	−
rfaF	Rd_2	Heptosyl II transferase	+	−
rfaG	Rd_1	Glucosyl I transferase (?)	+	−
galU	Rd_1	UDP glucose pyrophosphorylase	−	−
galE	Rc	UDP galactose epimerase	−	−
rfaP	Rc	Inability to transfer P of ATP to heptose	+	−
rfaI	Rb_3	Galactose I transferase	+	−
rfaJ	Rb_2	Glucose II transferase	+	−
rfaK	Rb_1	*N*-acetyl glucosamine transferase	+	+/−
rfaL	Ra	Transfer of O-antigen from lipid antigen		
rfbT	Ra	Carrier to complete core	+	−
rfc	SR	O-antigen polymerase	+	+[b]
rfb H, G, F, K, M, L, A, B, D, N	Ra	Biosynthesis of O-antigen	−	−

[a] Question marks indicate uncertainty as to the nature of the defect.
[b] One O-antigen unit per LPS molecule.

smooth LPS, a high proportion (up to 40 or 50%) of LPS molecules are of the Ra and the SR chemotypes. In general, LPS chemotypes with less than a complete core are not found in enteric, wild-type LPS. It is also noteworthy that, in some enterics such as *Escherichia coli*, the lengths of the O-antigen polymers (i.e., the number of O-antigen repeating units per core and lipid A molecule) vary in a rather predictable way resulting in one or two clusters of similarly sized LPS molecules. This phenomenon is less readily apparent in the *Salmonella* smooth LPS (Fig. 2).

Structure of LPS from nonenteric bacteria. In contrast to the extensive studies on the LPSs from enteric organisms, the structure and biochemical composition of the LPSs of nonenteric organisms, with the exception of *Neisseria meningitidis* and *Bordetella pertussis* (21, 32), have only recently received the full attention of investigators. However, interest in the role of LPS in the virulence of a variety of nonenteric pathogens has resulted in considerable new information on LPS. Perhaps one of the most striking observations to emerge is that the presence of O-antigen is rare as compared with the enteric organisms. For instance, the LPSs of *Haemophilus influenzae*, *B. pertussis*, *N. meningitidis*, and *Neisseria gonorrhoeae*, to mention a few, lack O-antigen (Fig. 5) (17, 20, 24, 47, 50, 51). Among these pathogens, there is some indication that biochemical heterogeneity of the core sugars has evolved as it has for the O-antigens of enteric bacteria. Like the variation in O-antigen composition, the variation in core phenotype is reflected in serological specificity (2, 26, 32, 38, 42, 50). Considering the nonvirulence of rough *Salmonella* mutants, it is remarkable that the virulence of nonenteric mucosal pathogens does not depend on the presence of O-antigens.

There are data to indicate that the LPSs of the nonenteric pathogens are biologically similar to and share the same general architecture with the LPSs of the enteric bacteria (4, 20, 24, 39, 41). The lipid A's of many of these organisms cross-react serologically with the lipid A of *Salmonella* sp. (P. Hitchcock, unpublished data). These LPSs are also biologically active in the *Limulus* amoebocyte lysate assay (a bioassay in which the hemolymph of the horseshoe crab *Limulus polyphemus* is incubated with lipid A or LPS; a positive reaction occurs when the hemolymph gels after incubation) and are toxic in the classic endotoxin assays such as the rabbit pyrogenicity assay (rabbits develop a biphasic fever [>40°C] within 1 and 3 h after intravenous injection of lipid A or LPS), the chick embryo lethality assay, and the mouse lethality assay (reviewed in reference 6). Furthermore, the KDO oligomer seems to be antigenically cross-reactive with the KDO of the Re LPS (P. Hitchcock, unpublished data). Recently, a gene(s), which encodes an enzyme(s) responsible for adding a *Chlamydia*-specific, periodate-sensitive LPS epitope (presumed to be a sugar and defined by reactivity with a monoclonal antibody [4]) has been cloned and expressed in *E. coli* K-12, indicating that lipid A core constituents of strain K-12 are able to serve as acceptors for the chlamydial sugar (37). In fact, there is a great deal of evidence to suggest that the LPSs of the nonenteric pathogens are quite similar to those of the enteric organisms, except that the O-antigen is absent.

One remarkable difference has been observed, however, and merits discussion at this point. The LPSs of two bacteria which are obligate intracellular parasites, *Chlamydia trachomatis* and *Coxiella burnetii*, as well as those of some phototropic bacteria are not endotoxins (10; reviewed in reference 31; P. Hitchcock and P. H. Mäkelä, unpublished observations). That is to say, they are not toxins in the rabbit pyrogenicity, chick embryo lethality, and mouse lethality assays. Although these LPSs are structurally analogous to other endotoxic LPSs, variation in lipid A appears to be responsible for the nontoxic properties of the former (31, 39).

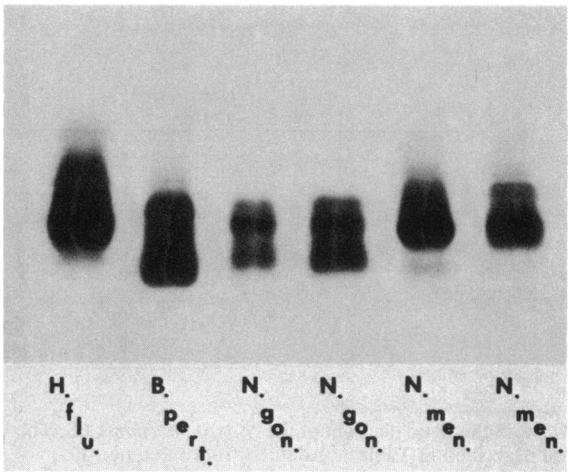

FIG. 5. Silver-stained sodium dodecyl sulfate-polyacrylamide gel (18%) containing 2 μg of LPS per lane from *H. influenzae* (H.flu), *B. pertussis* (B.pert.), *N. gonorrhoeae* FA1090 (N.gon.) extracted from samples having the nonpiliated and piliated transparent phenotypes, respectively. *N. meningitidis* 981 and slat y (N.men.). The LPS preparations were extracted by the method of Westphal et al. (55).

LPS nomenclative implications. Perhaps one of the most conceptually attractive features of endotoxin research is the fact that LPS molecules can be obtained from a diverse spectrum of gram-negative organisms; the majority of these LPSs manifest remarkably similar biochemical structures and biological activities. In this respect, the active component of LPS, namely lipid A, is highly conserved in its chemical structure and in its antigenic cross-reactivity (45). Based on this structural similarity of the lipid A, most LPSs are assumed to have a common mode of action. At the same time, each individual organism maintains its LPS structural and immunological identity on the basis of the saccharides (core and O-antigen) attached to lipid A. It is to be expected that the biochemical nature of the polysaccharide will modulate the biochemical activity of lipid A and, in certain instances, that the polysaccharide will manifest activity of its own (52). Finally, since it is now recognized that LPS is heterogeneous, the contribution of the extraction method to the biochemical composition of the resulting LPS molecule and to the presence of non-LPS contaminants must also be considered.

Of importance therefore, particularly with respect to the latter point, the term lipopolysaccharide and its abbreviation, LPS, should be reserved for purified bacterial extracts which are reasonably free of detectable contaminants, particularly protein, which, in some assays, can manifest biological activities similar to those of LPS (reviewed in reference 12). Products of extraction procedures which result in macromolecular complexes of LPS, protein, and phospholipid (e.g., those generated by Boivin-TCA [1], butanol [35], and EDTA [25] extraction methods) should retain the designation endotoxin. It should be noted that not all bacterial LPSs are endotoxins. Under no circumstances should a given LPS preparation, for example reference endotoxin, be designated solely on the basis of its production by a partic-

ular manufacturer, without reference to the strain of bacteria from which the preparation was derived.

Accompanying the clarification of the nature of the truncated LPS chemotypes of rough mutants and with the development of methods to purify these LPSs to homogeneity (7), several terms have been proposed to designate these preparations more precisely. The overall objective has been to distinguish these mutant LPSs from the more typical O-antigen-containing, LPS preparations. The terms glycolipid and lipooligosaccharide have been introduced to specify the rough LPSs of enteric mutants and the rough LPSs of nonenteric mucosal pathogens, respectively. While it is clear that the use of these terms to designate specific LPS preparations has certain advantages, there are also attendant problems.

The term glycolipid was originally introduced to describe the deep rough LPS mutants of *Salmonella* spp. However, this term has not been generally embraced. Of particular concern is the fact that this term is currently used for the glycosylated lipids of eucaryotic cells (primarily sialogangliosides) as well as for the surface glycolipids of acid-fast procaryotic cells of the *Mycobacterium* spp. (reviewed in reference 3). Thus, this term does not appear to be an appropriate or specific designation for LPS of the rough chemotype.

More recently, the term lipooligosaccharide has been introduced into the literature to denote the rough LPS of *N. gonorrhoeae*, *N. meningitidis*, and *H. influenzae* (47, 51; T. Inzana, M. F. Tosi, S. L. Kaplan, E. O. Mason, and R. P. Williams, unpublished data). The arguments advanced in favor of the term are that it is more structurally correct to refer to the rough LPS molecules as lipooligosaccharides rather than the traditional LPS. However, the biochemical structure of these molecules has not been fully elucidated. Definitions of the terms oligosaccharide (16) and polysaccharide (15) indicate a difference in the numbers of sugars which are released when the molecules are hydrolyzed, oligosaccharides yielding 2 to 10 and polysaccharides yielding more than 10 sugars. The question of the accuracy of the term oligosaccharide versus polysaccharide cannot be totally resolved until structural analyses of these LPSs are completed. Most importantly, as with the term glycolipid, the term lipooligosaccharide is used in another context; it has been extensively used by investigators to describe the species-specific lipid and sugar antigens of *Mycobacterium* spp. (13, 14).

The most compelling consideration, however, attendant to the specific issue of the term lipooligosaccharide versus the term lipopolysaccharide is that having two terms suggests that these are fundamentally distinct molecules, and there is no reason to believe that this is the case. The primary advantage of the term rough or R LPS is that it connotes a biologically active molecule, unique to the gram-negative bacterial outer membrane, which lacks O-antigen. Also, it is well recognized that there is structurally heterogeneity in enteric smooth LPS preparations. Within the heterogeneous population, LPS molecules with various numbers of O-antigen units, there is a significant percentage of LPS molecules of the Ra and SR chemotypes which might, by the appropriate definition of these terms, be called lipooligosaccharides. Thus, if the lipooligosaccharide terminology were to be universally applied, then native smooth LPSs might need to be redesignated as lipopolysaccharide-lipooligosaccharide complexes. While perhaps more biochemically precise, this cumbersome terminology would do little to reduce confusion among investigators working in this field.

The term lipopolysaccharide, modified by either the adjective smooth or rough, is so strongly identified with the lipid-sugar molecules which form the outer leaf of the gram-negative outer membrane that it might be considered redundant to say "lipopolysaccharide of gram-negative bacteria." This term as well as the extant nomenclature (which includes more than the 40 terms mentioned here) is known not only to lipopolysaccharidologists but also to microbiologists and health science professionals whose primary interests lie outside the area of LPS. To introduce additional terms encourages confusion and misunderstanding. As in any other discipline, accessibility is affected by the complexity of the jargon associated with it. It is, therefore, desirable to minimize the terminology used to describe these phenomena. That is not to say that one should not consider introducing new, more accurate, and necessary terms into the LPS dictionary; however, at the very least, it seems premature to rename the LPS of nonenteric mucosal pathogens, and at the very worst, this practice will likely be the source of confusion inside and outside the discipline.

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