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Conjugation of Meningococcal Lipopolysaccharide R-Type Oligosaccharides to Tetanus Toxoid as Route to a Potential Vaccine Against Group B *Neisseria meningitidis*†

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Oligosaccharides were obtained by the mild acid hydrolysis of the lipopolysaccharides from a number of different strains of *Neisseria meningitidis*, serotypes L2, L3, L4, L5, and L10. The dephosphorylated oligosaccharides were conjugated to tetanus toxoid as their 2-(4-isothiocyanatophenyl)-ethylamine derivatives, which resulted in the incorporation of from 18 to 38 oligosaccharides per molecule of tetanus toxoid. When injected in rabbits, the conjugates produced oligosaccharide-specific antibodies which were predominantly serologically specific but which also exhibited some cross-reactivity. These serological results can be attributed to regions of structural dissimilarity and similarity within the oligosaccharides. The oligosaccharide-specific antibodies were also lipopolysaccharide serotype specific, thus indicating that the oligosaccharides are the determinants associated with this serotype specificity. Consistent with the serological results, the conjugate antisera were bactericidal for the homologous serotype meningococcal organisms and in some cases for heterologous serotype organisms.

Because of the poor immunogenicity in humans (20) of the group B polysaccharide of *Neisseria meningitidis*, the use of other vaccines based on the subcapsular protein serotype antigens of the group B organisms is being explored (3, 23). The meningococcal lipopolysaccharides (LPS) have been implicated in the immune response to natural infection (4), and 13 different LPS serotypes have now been identified (14, 17, 21, 22). Some of the serotype determinants are shared to a certain extent among the LPS, thus producing a complex serological response (14, 21). We have shown that the determinants responsible for this serotype specificity are located in the low-molecular-weight (R-type) oligosaccharides of the LPS (8, 9; see below). In addition, regions of structural difference and similarity have been identified in the oligosaccharides which are consistent with the variety of serotypes found in the meningococcal organisms (8, 9). Because of the adverse reactions encountered in using LPS as vaccines, our strategy was to conjugate a number of these oligosaccharides (serotypes L2, L3, L5, and L10) to tetanus toxoid to yield a group of more acceptable immunogens capable of raising protective antibody against a wide range of group B organisms.

MATERIALS AND METHODS

Crystalline bovine serum albumin (BSA) was obtained from Schwarz-Mann, Orangeburg, N.Y., and tetanus toxoid was a gift from W. Hankins of Connaught Laboratories, Swiftwater, Pa. and was additionally purified with a Sephadex G-100 column (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline (PBS) at pH 7. Only the fraction in the void volume of the column was used in the conjugation experiments. Sodium cyanoborohydride and 2-(4-aminophenyl)-ethylamine were obtained from Aldrich Chemical Co., Milwaukee, Wis.

Growth of the organisms. The strains and serotypes of *N. meningitidis* used in this study, with groups given in parentheses, were 604 (A), L10(d); 608 (B), L3(d), L9(m); 981 (B), L5(d); 2241 (C), L2(d), L3(m); 89I (C), L4(d); 60E (C), L3(d), L4, L7(m); and Slaterus (Y) L3, L9(d), L7(m) (d is a dominant and m is a minor determinant). The LPS serotyping was kindly performed by Wendell D. Zollinger of the Walter Reed Army Institute of Research, Washington, D.C. by his procedures (21, 22). Prototype strains M986 (B), L3(d), L2, L7(m); M1080 (B), L3(d), L4, L7, L8(m); and M1011 (B), L3(d), L7(m) were obtained from C. E. Frasch of the Bureau of Biologics, National Institutes of Health, Washington, D.C. Inocula were prepared from lyophilized cultures which were grown overnight on sheep blood agar plates at 37°C in candle jars. Cellular proceeds from five or six blood agar plates were dispersed in 1 liter of *Neisseria* Chemically Defined Medium (General Biochemicals Inc., Chagrin Falls, Ohio) previously sterilized by passage through 0.22-μm filters (Millipore Corp., Bedford, Mass.) and agitated in a 4-liter baffled flask at 37°C for 7 h. At this time, the inoculum was transferred to a 25-liter New Brunswick Microferm containing 20 liters of *Neisseria* Chemically Defined Medium. Aeration and agitation were maintained at 25 liters/min and 200 rpm, respectively. After 18 h of growth at 37°C, cells were killed by the addition of 1% Formalin and were harvested by centrifugation.

Isolation of the LPS. The LPS from the different serogroups were isolated by a modified phenol extraction technique as previously described (8). In this technique, the LPS was obtained from the aqueous extract in yields of approximately 1% (depending on the serogroup) based on the dry weight of the organisms. Filtration and lyophilization of this solution yielded the crude LPS, which was purified by dissolution in water and by repetitive centrifugation of this solution at 105,000 × g for 12 to 16 h. The purity of the LPS preparations was monitored by the carbocyanine dye assay method (7) as previously described (8).

Isolation and modification of the LPS core oligosaccharides. The core oligosaccharides were obtained by heating the LPS

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in 1% acetic acid at 100°C for 2 h and were purified by gel filtration on a Sephadex G-25 column (8). The cores were dephosphorylated with 48% hydrogen fluoride as previously described (8) and were separated from the released phosphate and ethanolamine by further gel filtration on a Sephadex G-25 column. The oligosaccharides were lipid-free as previously established by the absence of multiple signals in the 18 to 25 ppm region of their ^{13}C nuclear magnetic resonance spectra (9a). These signals are diagnostic for the presence of lipid.

Preparation of oligosaccharide-protein conjugates. The dephosphorylated oligosaccharides were conjugated through their reducing terminal KDO (2-keto-3-deoxyoctonic acid) residues to BSA and tetanus toxoid directly by reductive amination (18) and indirectly by the prior preparation of oligosaccharide-2(4-isothiocyanatophenyl)-ethylamine derivatives (15, 19). The methods employed in the preparation and purification of these conjugates have been previously described in detail elsewhere (10). The protein content of the conjugates was determined by the method of Lowry et al. (13), and the amount of oligosaccharide was determined by the phenol-sulfuric acid method (2). BSA and D-glucose were used to construct the standard curves for protein and oligosaccharides, respectively.

Preparation of oligosaccharide-affinity chromatography column. The 2-(4-isothiocyanatophenyl)-ethylamine derivative of the dephosphorylated group Y (L3) core (25 mg) was shaken overnight at room temperature with 20 ml of aminoethyl–Bio-Gel P-300 (5, 19) suspended in PBS at pH 9.0. The modified gel was washed continuously with water, and the amount of oligosaccharide linked to the gel was determined by the phenol-sulfuric method (2) (580 μg of oligosaccharide per ml of gel). The excess free amino groups on the gel were acetylated by the dropwise addition of acetic anhydride (2 ml) to a dispersion of the gel in a stirred saturated aqueous sodium bicarbonate solution (100 ml). The resultant gel was washed with water and gave a negative 2,4,6-trinitrobenzene sulfonic acid test (1) for free amino groups, indicating that all the free amino groups had been acetylated.

Immunization procedures. New Zealand white rabbits (approximate weight, 2 to 3 kg) were immunized in the footpads with 30 to 50 μg of the various conjugates dissolved in 100 μl of PBS emulsified in 100 μl of Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). Two immunizations were given 21 days apart, and the rabbits were bled before immunization and 10 days after the last injection. In some cases, the rabbits were test bled 10 days after the first injection. A control rabbit antiserum was also made by the above procedure with Freund adjuvant alone as the immunogen.

Immunodiffusion. Double radial immunodiffusion (16) was performed in 0.9% agarose gels in PBS containing 2% polyethylene glycol (molecular weight, 4,000).

Passive hemagglutination. Passive hemagglutination tests were carried out in microtiter plates (Linbro Division, Flow Laboratories, Inc., Hamden, Conn.). Horse erythrocytes (1% suspension in PBS) were incubated with alkali-treated LPS preparations (0.25 N NaOH at 56°C for 60 min) at concentrations of 30 μg of LPS per ml for 90 min at 37°C. The coated erythrocytes were then washed five times with PBS. For titration of antisera, 25 μl of 0.5% erythrocyte suspension was mixed with 25 μl of serum (twofold dilutions) and left at room temperature for 2 h. The serum titer was the highest dilution of the serum giving maximal strong hemagglutination. A previously prepared (8) rabbit antiserum to whole group 604A (L10) meningococcal organisms was used as the reference antiserum.

Inhibition tests were carried out in the following way. Antisera (25 μl) at a dilution four times lower than maximal giving strong hemagglutination were incubated with 25 μl of inhibitor solution for 60 min at room temperature. After incubation, 25 μl of 0.5% horse erythrocytes coated with LPS was added, and incubation was repeated for an additional 2 h at room temperature.

Enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assays were performed by the method of Ito et al. (6) except that 0.10 ml of LPS (100 μg/ml) was used to coat the wells of E.I.A. microtitration plates (Linbro Division, Flow Laboratories, Inc.) overnight at ambient temperature. Alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (heavy and light chains; ZYMED Laboratories, Burlingame, Calif.) was used at a dilution of 1:1,000. Optical densities were read with a Titertek Multiskan (Flow Laboratories, Mississauga, Ontario, Canada) 40 min after the addition of substrate (nitrophenol phosphate, 1 mg/ml; Sigma Chemical Co., St. Louis, Mo.). The results of each assay were corrected by an internal standard (reference serum) which was a 1:4,000 dilution of anti-M981 (LPS) serum (rabbit no. 144).

Bactericide assay. Pre- and postimmune rabbit sera were heated at 56°C for 30 min. After an initial fivefold dilution, twofold dilutions of the sera were made in Hanks balanced salt solution, pH 7.2, containing 0.1% gelatin (HBSG). A pool of normal sera obtained from a number of 4-week-old rabbits was used as a source of complement. Meningococci were grown on GC medium (11) for 4 to 5 h at 37°C in 5 to 10% CO_2. A sample of organisms (0.08 ml, approximately 1.5×10^4 CFU/ml of HBSG) was added to each mixture of 0.10 ml of serum dilution and 0.02 ml of the complement source. Controls consisted of 0.08 ml of the organisms added to either 0.12 ml of HBSG, 0.10 ml of serum dilution and 0.02 ml of HBSG, or 0.10 ml of HBSG and 0.02 ml of complement. The mixtures were kept in a 37°C water bath for 30 min, and then 0.05 ml of each mixture was plated on GC medium. The plates were incubated at 37°C for 24 h in 5 to 10% CO_2. The reciprocal of the serum dilution giving 50% killing of meningococci was considered to be the titer of the serum.

RESULTS

Conjugation of oligosaccharides to protein. Oligosaccharide cores were obtained by the mild acid hydrolysis of the L2, L3, L5, and L10 serotype LPS, and before conjugation their ethanolamine phosphate substituents were removed with aqueous hydrogen fluoride (8). An attempt was made to covalently link the oligosaccharides (approximate molecular weight, 1,500) to tetanus toxoid via the hemiketal of their terminal 2-keto-3-deoxyoctulosonic acid residues by the reductive amination procedure (18). The stoichiometry of the best of these conjugates (Table 1; molar ratio of oligosaccharide to tetanus toxoid, 5:1) demonstrates that even after protracted reaction times, only relatively poor incorporation of the oligosaccharides was achieved. Therefore, the conjugates were linked by a previously published indirect procedure involving the prior formation of their 2-(4-aminoisocyanatophenyl)-ethylamine derivatives (15, 19). This type of conjugation has previously been applied to oligosaccharides terminating in glycoses through their hemiacetal group (18, 19) and in sialic acid through its hemiketal group (10). More recently, this method has also been applied to the conjugation of the analogous oligosaccharides obtained from the LPS of *Neisseria gonorrhoeae* (12). All the meningococcal

TABLE 1. Stoichiometry of the protein conjugates of the oligosaccharides from the meningococcal LPS

Serogroup and serotype	Protein	Molar ratio of oligosaccharide to protein	
		Reaction mixture	Conjugate
604A (L10)[a]	Tetanus toxoid	220:1	18:1
981B (L5)[a]	Tetanus toxoid	350:1	38:1
2241C (L2)[a]	Tetanus toxoid	380:1	22:1
Y (L3, 9)[a]	Tetanus toxoid	225:1	30:1
Y (L3, 9)[a]	BSA	230:1	30:1
Y (L3, 9)[b]	BSA	140:1	5:1

[a] Using the active 2-(4-aminocyanatophenyl)-ethylamine derivative.

[b] Reductive amination; time of reaction, 21 days.

oligosaccharides were successfully linked to tetanus toxoid by this procedure, the incorporation of oligosaccharide being much improved (Table 1) over the direct coupling method.

Immunological properties of the oligosaccharide-tetanus toxoid conjugates. The oligosaccharide-tetanus toxoid conjugates were used as immunogens in rabbits, and the antisera were evaluated by immunodiffusion and passive hemagglutination. The immunodiffusion experiments (Fig. 1) with the L2, L3, and L10 conjugates indicate that the L2 and L10 conjugates produced antibodies that gave precipitin lines against their homologous LPS and none against the heterologous LPS. In the case of the L3 conjugate, a weaker precipitin line was obtained with its homologous LPS, and a cross-reaction was detected with the L4 LPS.

More extensive and sensitive serological experiments by the passive hemagglutination technique were performed with the antisera to the L2, L3, L5, and L10 conjugates and the alkali-treated L2, L3, L4, L5, and L10 LPS. The results (Table 2) are consistent with the immunodiffusion data in that the antisera are largely serotype specific, the antisera to the L2 and L10 conjugates being the strongest. However, passive hemagglutination experiments were also able to detect additional weaker cross-reactions and a previously undetected strong cross-reaction between the L3 LPS and antisera to the L2 conjugate. This cross-reacting antibody could be removed from the L2 antisera by using an L3 oligosaccharide affinity column. By the introduction of the L5 LPS antigen into these experiments, strong cross-reactions were also detected between the L5 LPS and the L2 and L10 conjugate antisera. This latter cross-reaction was also detectable in the converse situation.

Inhibition experiments by the passive hemagglutination technique were carried out on the antiserum to the L10 conjugate and an antiserum to the homologous organisms. The effects of the different inhibitors (Table 3) indicate clearly that the L10 oligosaccharide is a good inhibitor of both antisera. Except for the L5 oligosaccharide and LPS, which exhibited detectable but weak inhibitory properties to both antisera, all the serotype oligosaccharides and LPS exhibited no detectable inhibitory properties. It is of interest that the lipid A moiety of the L10 LPS was as strong an inhibitor of the antisera to the L10 oligosaccharide and homologous organisms as the L10 LPS itself was.

Bactericidal activity of antisera to the oligosaccharide conjugates. The bacteridical activities of the conjugate antisera against both homologous and heterologous serotype organisms are shown in Table 4. The equivalent levels of homologous and cross-reacting antibodies in each conjugate antiserum were determined by enzyme-linked immunosorbent assay with the different serotype LPS as antigens (Table 4). All the conjugate antisera exhibited homologous serotype bactericidal activity, the activity increasing with increasing antibody levels. This was also generally true for the heterologous bactericidal activity; the antisera containing the highest levels of cross-reacting antibodies also proved to be the most bactericidal for heterologous serotype organisms. From Table 4 it would also appear that the extent of cross-reactivity among the different conjugate antisera is also related to the level of homologous antibody in the antiserum, the L10 conjugate antiserum being the most cross-reactive. One notable exception is the failure of the L5 conjugate antisera to exhibit bactericidal activity against the serotype L2 organisms, despite the presence of significant levels of antibodies cross-reacting with the L2 LPS. Also of interest is the observation that the L3 conjugate antiserum, which exhibited the lowest homologous antibody and bactericidal titers of all the conjugate antisera, gave a higher bactericidal titer against the heterologous L2 serotype organisms than it did with its homologous organisms.

DISCUSSION

Because of the poor immunogenicity of the group B meningococcal polysaccharide in humans (20), the alternate strategy of using subcapsular serotype protein antigens as human vaccines, either alone or in combination with the group B capsular polysaccharide, has recently been explored (3, 23). Another subcapsular antigen that is also involved in the serology of the meningococcal organisms is the LPS. Zollinger and Mandrell (21) have shown that the LPS are serotype antigens and have identified eight different LPS serotype determinants among the group B organisms. Some of these determinants are shared to a certain extent among

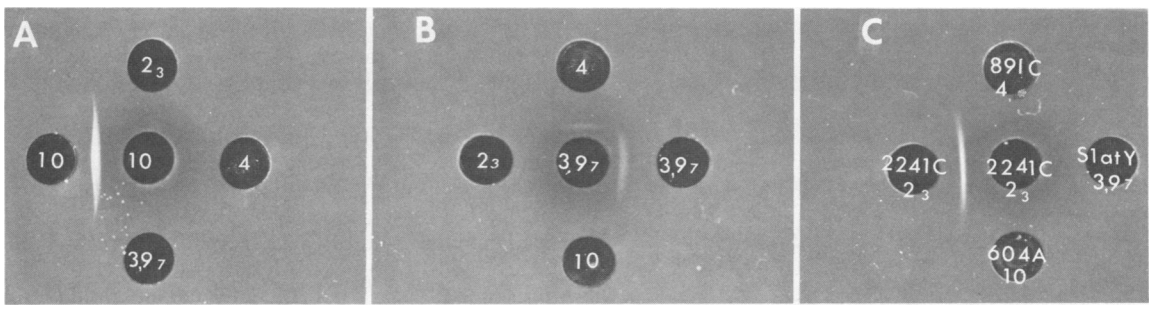

FIG. 1. Immunodiffusion of the serotype L2, L3 and L10 conjugate antisera (center wells) against the serotype L2, L3, L4, and L10 meningococcal LPS (outer wells).

TABLE 2. Passive hemagglutination titers of sera to conjugates

Conjugate antiserum[a]	Rabbit	Hemagglutination titer for strain[b]:				
		604A (L10)	981B (L5)	2241C (L2)	89IC (L4)	Y (L3)
604A (L10)	141	8,000	256	8	2	4
604A (L10)	142	4,000	128	4	4	32
2241C (L2)	145	32	1,000	2,000	32	128
2241C (L2)	146	16 12[c]	4,000 3,200[c]	8,000 6,300[c]	64 32[c]	512 4[c]
981B (L5)	143	128	512	32	16	32
Y (L3)	147	8	16	16	32	256

[a] Titers of preimmunization sera and a rabbit antiserum made to Freund adjuvant alone were <4.
[b] Titers for erythrocytes sensitized with the LPS from the strain indicated.
[c] Titers after passage of the L2 oligosaccharide conjugate antiserum through an L3 oligosaccharide affinity column.

the LPS, thus producing a complex serological response. The meningococcal LPS are of the R type, having low-molecular-weight core oligosaccharides, and it was conjectured that the oligosaccharides were the determinants associated with serotype specificity (8, 9). This is amply confirmed in all the serological experiments described herein.

Because LPS are too toxic to use as immunogens, it was decided to conjugate the serotype oligosaccharides to a nontoxic and immunologically acceptable protein carrier, i.e., tetanus toxoid. This protein has been used as a carrier in the synthesis of numerous recent polysaccharide conjugates (10). The use of reductive amination to effect a direct coupling between the oligosaccharide and tetanus toxoid via the hemiketal of the terminal KDO residue resulted in a relatively poor incorporation of the oligosaccharides (Table 1). However, the use of a previously published procedure in which the oligosaccharides were converted to their 2-(4-aminoisocyanatophenyl)-ethylamine derivatives before conjugation (19) resulted in much-improved incorporation of the oligosaccharides (Table 1). Lambden and Heckels (12) have also recently reported the conjugation of an analogous gonococcal oligosaccharide to protein by this method.

TABLE 3. Inhibition of passive hemagglutination using horse erythrocytes coated with 604A (L10) LPS

Inhibitor	Inhibitory concn (μg/ml)[a] of antiserum:	
	To whole serotype L10 organisms	To L10, oligosaccharide conjugate
L10 oligosaccharide	150	15
L10 oligosaccharide (hydrogen fluoride treated)	150	15
L5 oligosaccharide	2,500	1,250
L2, L3,9, and L4 oligosaccharides	2,500	2,500
L10 serotype LPS (base treated)	30	0.5
Lipid A from L10 serotype LPS	40	0.5
L5 serotype LPS (base treated)	500	500
L2, L3,9, and L4 LPS (base treated)	5,000	5,000

[a] Concentration giving 100% inhibition.

The serological experiments with the above oligosaccharide conjugates used as immunogens in rabbits indicated that each conjugate was able to elicit oligosaccharide-specific antibody, some more effectively than others. In these experiments, it was noticeable that the conjugate prepared from the serotype L3 oligosaccharide consistently produced the lowest levels of antibodies with oligosaccharide specificity. As shown by immunodiffusion and passive hemagglutination experiments, the major LPS serotype specificity was retained in the conjugate antisera, despite the fact that the oligosaccharides had been modified by the removal of their ethanolamine phosphate substituents before conjugation. Thus, the major serotype determinants must still be located in the modified oligosaccharides. This was also confirmed in the case of the oligosaccharide obtained from the L10 serotype LPS by inhibition experiments. Both the original L10 oligosaccharide and the dephosphorylated L10 oligosaccharide were equally strong inhibitors of the agglutination of the LPS-coated erythrocytes with antisera to both the L10 oligosaccharide conjugate and the homologous organisms. Both the base-treated L10 LPS and its lipid A moiety were also good inhibitors of the above agglutination. That the lipid A moiety had inhibitory properties equivalent to those of the L10 LPS and its oligosaccharide was unexpected and was probably due to the incomplete removal of the oligosaccharide from the lipid A moiety during the mild acid hydrolysis of the LPS (8, 9).

The structure of the modified oligosaccharide obtained from the group Y serotype L3 LPS has been elucidated (9a) and is shown in Fig. 2. The oligosaccharide has a lacto-*N*-neotetraose branch and a shorter branch terminating in 2-acetamido-2-deoxy-α-D-glucopyranosyl residues. This latter disaccharide branch is also a structural component of the L10 oligosaccharide (L. Kenne and H. J. Jennings, manuscript in preparation), and therefore, because of the high degree of serological specificity exhibited by the antisera of the L3 and L10 oligosaccharide conjugates (Fig. 1, Tables 3 and 4), the serotype specificity of the L3 oligosaccharide probably resides in its lacto-*N*-neotetraose branch.

Although all the oligosaccharide conjugate antisera were strongly serologically specific, strong cross-reactivities were also detected, some of which could be explained on the basis of previously proposed determinants (14, 17, 21, 22). The presence of a common L3 determinant could be responsible for the cross-reaction between the group Y LPS and the

TABLE 4. Bactericidal activities of oligosaccharide conjugate antisera against homologous and heterologous meningococcal organisms

Conjugate antiserum	Rabbit	Bactericidal activity against strain: 604A (L10)		981B (L5)		2241C (L2)		Y (L3)		608B (L3)		89IC (L4)	
		Titer[a]	Antibody level[b]	Titer	Antibody level	Titer	Antibody level	Titer	Antibody level	Titer	Antibody level	Titer	Antibody level
604A (L10)	141	256	16,000	16	250	8	250	0	100	0	100	16	100
	142	512	32,000	64	4,000	64	8,000	4	500	0	100	64	8,000
	275	1,024	32,000	32	2,000	64	8,000	0	100	0	100	64	4,000
981B (L5)	143	2	100	64	4,000	0	1,000	0	100	0	100	0	100
	144	8	250	512	32,000	0	1,000	0	100	0	100	0	100
2241C (L2)	145	0	100	8	500	16	1,000	4	100	0	100	0	100
	277	0	100	32	1,000	128	4,000	0	100	0	100	64	2,000
Y (L3,9)	147	0	100	0	100	32	500	4[c]	250	0[d]	100	16	250

[a] Fold increase above preimmunization sera. Titers of preimmunization sera ranged from 1:10 to 1:20, and an equivalent titer was also obtained with a rabbit antiserum made to Freund adjuvant alone.

[b] Measured by enzyme-linked immunosorbent assay and expressed as the reciprocal of the highest serum dilution which gave an optical density of ≥0.25. Both preimmunization sera and a rabbit antiserum made to Freund adjuvant alone gave an optical density of <0.1.

[c] Strain 60E (C) (L3) gave a bactericidal titer of 16.

[d] Strains M986 (B), M1011 (B), and M1080 (B), all containing L3 major determinants, exhibited no increase in bactericidal titer.

antiserum specific for the oligosaccharide obtained from the group 2241C LPS. However, no common determinants have previously been identified to account for the very strong cross-reactivities exhibited by groups 604A, 981B, and 2241C LPS and their respective oligosaccharide conjugate antisera.

Both serotype-specific and cross-reactive antibodies in the conjugate antisera are involved in bactericidal activity against meningococcal organisms, the bactericidal titer being in most cases proportional to the antibody levels (Table 4). Serotype-specific bactericidal activity was detected in all the oligosaccharide conjugate antisera, although the titer against the group Y serotype L3 organisms was low compared with the others. This is consistent with the low antibody titers given by the L3 oligosaccharide conjugate antiserum. Interestingly, this L3 serotype-specific antiserum gave a higher bactericidal titer against the heterologous 2241C (L2) and 89IC (L4) organisms. Heterologous bactericidal activity occurred mainly when the concentrations of cross-reactive antibodies were the highest (Tables 3 and 4), e.g., antisera to the L10 oligosaccharide conjugate were bactericidal for groups L981B, 2241C, and 89IC organisms. In none of these organisms has the L10 determinant been previously detected.

Another interesting observation that can be made from the bactericidal experiments is that the L3 oligosaccharide conjugate antiserum was not bactericidal for all organisms carrying the L3 determinant. In fact, from the bactericidal assays listed in Table 4 one could specifically associate lack of bactericidal activity with group B organisms carrying the L3 determinant. It is not known whether the group B capsular polysaccharide is involved in any possible mediation of bactericidal activity involving the L3 determinant. However, such an association is tenuous at present because it is based on only a limited number of strains and also because even the bactericidal activity against homologous group Y organisms was not high.

The above study confirms the serological complexity associated with the meningococcal LPS and provides evidence that this complexity is due to regions of structural similarity in the individual LPS R-type oligosaccharides acting as common cross-reactive determinants. However, despite this cross-reactivity detected in various degrees in all the conjugate antisera, the tendency of the antisera to be mainly serologically specific indicates that a true serotyping system based on unique structural determinants within the R-type oligosaccharides is still possible. Until these specific determinants have been structurally defined, however, this serotyping system would have to be based on conjugate antisera previously absorbed with heterologous LPS containing common determinants. Another approach would be to produce monoclonal antibodies to the meningococcal LPS

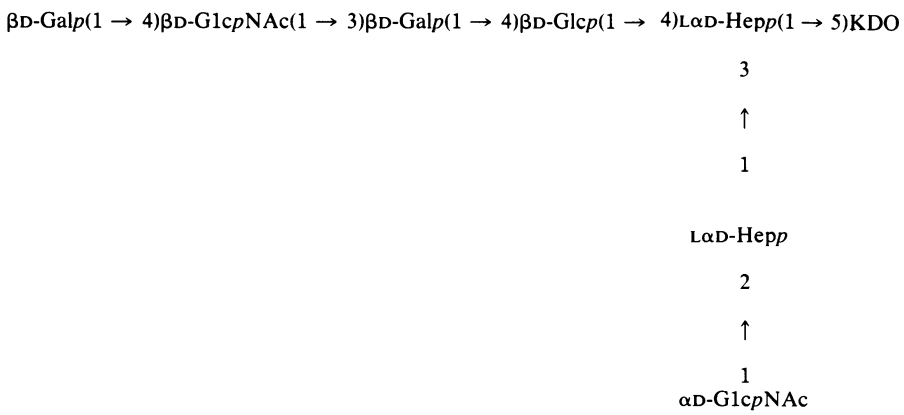

FIG. 2. Structure of the dephosphorylated oligosaccharide obtained from the serotype L3 LPS.

by using cells previously screened to eliminate those producing antibody to shared determinants. From the perspective of using these oligosaccharide conjugates as human vaccines, the high degree of serotype specificity exhibited by the conjugate antisera would probably necessitate the use of a number of oligosaccharide conjugates to provide protection against a wide range of meningococcal group B organisms.

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