Cross-Reactive Polyclonal Antibodies to the Inner Core of Lipopolysaccharide from *Neisseria meningitidis*

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Sera from mice immunized with native or detergent-extracted outer membrane vesicles derived from lipopolysaccharide (LPS) mutant 44/76Mu-4 of *Neisseria meningitidis* were analyzed for antibodies to LPS. The carbohydrate portion of 44/76Mu-4 LPS consists of the complete inner core, Glcâ1→4[GlcNAc â1→2Hep â1→3]Hep â1→5KDO[â4→2KDO]. Immunoblot analysis revealed that some sera contained antibodies to wild-type LPS which has a fully extended carbohydrate chain of immunotype L3,7, as well as to the homologous LPS. Sera reacted only weakly to LPS from 44/76Mu-3, which lacks the terminal glucose of the inner core. No binding to more truncated LPS was observed. Consequently, the cross-reactive epitopes are expressed mainly by the complete inner core. Dephosphorylation of wild-type LPS abolished antibody binding to LPS in all but one serum. Thus, at least two specificities of cross-reactive antibodies exist: one is dependent on phosphoethanolamine groups in LPS, and one is not. Detection of these cross-reactive antibodies strongly supports the notion that epitopes expressed by meningococcal LPS inner core are also accessible to antibodies when the carbohydrate chain is fully extended. Also, these inner core epitopes are sufficiently immunogenic to induce antibody levels detectable in polyclonal antibody responses. Meningococci can escape being killed by antibodies to LPS that bind only to a specific LPS variant, by altering the carbohydrate chain length. Cross-reactive antibodies may prevent such escape. Therefore, inner core LPS structures may be important antigens in future vaccines against meningococcal disease.

Meningococcal disease caused by *Neisseria meningitidis* (meningococci) is still a worldwide health problem. The clinical symptoms range in severity from a mild sore throat to acute meningococcemia which can quickly become fatal due to the rapid onset of circulatory collapse and multiorgan dysfunction (7). The most common presentation is acute purulent meningitis; less commonly, patients present with meningococcal septicemia or a combination of these two forms. The age groups that are most affected are children less than 4 years of age and teenagers.

Meningococci are divided into serogroups according to antigenic and structural differences in their anionic capsular polysaccharides (CPS) (17, 20, 27–29). Protective vaccines based on CPS from serogroups A, C, 135-W, and Y are available, but CPS from serogroup B is poorly immunogenic. A universal vaccine to protect against serogroup B meningococcal disease has yet to be developed, but a number of potential vaccine candidates are currently under intense investigation.

Lipopolysaccharide (LPS) is one of several potential vaccine candidates for two main reasons. First, LPS is the most abundant component of the gram-negative outer membrane and therefore is easily accessible to antibodies. Second, the level of bactericidal antibodies in serum correlates with protection against meningococcal disease (14). Both human and murine antibodies to LPS have been found to be bactericidal in vitro. Furthermore, monoclonal antibodies (MAbs) to LPS of immunotype L3,7 have also been found to be protective in the baboon model (35, 36). Consequently, antibodies to LPS may play an important role in the protection against meningococcal disease.

The major problem in using intact LPS as an immunogen is that it has undesirable endotoxic properties due to its lipid A portion. Removal of lipid A abolishes the immunogenicity of the carbohydrate chain. Conjugating the oligosaccharide portion of LPS to a protein carrier may restore the immunogenicity. Some conjugates have been found to induce bactericidal antibodies to LPS, whereas others have failed to do so (15, 19, 42–44). An alternative strategy is to detoxify LPS by removing the O-linked fatty acids from lipid A, either chemically (6, 32) or by introducing mutations in the lipid A biosynthesis genes (31, 39). Detoxified LPS from *Escherichia coli* J5 complexed with meningococcal outer membrane complexes has previously been shown to induce protection against gram-negative sepsis in an animal model (6).

Interestingly, vaccine formulations containing large amounts of fully active LPS in natural outer membrane vesicles (NOMV) have been administrated intranasally in humans without harmful side effects (12). In addition, intranasal immunization with NOMV was found to induce bactericidal antibodies to LPS (12). This suggests that the nasal mucosal surface is rather insensitive to the toxic activity of LPS. Intranasal immunization with preparations containing LPS may therefore represent an alternative strategy for investigating the immunogenicity of LPS epitopes, without the need for detoxification.

Meningococcal LPS is classified into 11 immunotypes (24,

tions under the United Kingdom Home Office Animals (Scientific Procedures) Act 1986.

46, 47), but only some of these (i.e., immunotypes L3,7 and L2) appear to be more prevalent among virulent strains (4, 21, 37).

Each immunotype is defined by its sugar moieties and phosphoethanolamine groups linked to either of the two heptose residues of the conserved inner core (Fig. 1) (10, 11, 13, 18, 22, 26, 33). A single strain can express more than one LPS immunotype, by altering the number of sugar residues attached to the conserved inner core (3, 25, 37). For example, immunotype L8 is a truncated version of both L3,7 and L1. Antigenic variation within the bacterial population of a single strain has been found to allow escape from bactericidal killing (4). Vaccine development using meningococcal LPS as an immunogen must therefore take into account the antigenic heterogeneity of LPS.

An ideal LPS-based vaccine should be able to induce antibodies that bind to all immunotypes. The inner core region contains structural motifs that are conserved among the various immunotypes. Therefore, antibodies against it could theoretically be cross-reactive.

We have been investigating the immune responses to the inner core structure of meningococcal LPS when integrated into NOMV or detergent-extracted outer membrane vesicles (DOMV). NOMV contain 25 to 50% (wt/wt) LPS relative to protein, whereas DOMV contain only 5 to 8%. Both OMV preparations were derived from an LPS mutant of serogroup B N. meningitidis strain 44/76 (Mu-4) which synthesizes only the complete inner core (2) (Fig. 1). In this study, we report the induction of polyclonal antibodies against the inner core structure that could also bind to LPS having a fully extended carbohydrate chain.

**Bacterial strains and growth conditions.** N. meningitidis strain 44/76 and its LPS mutants 44/76Mu-1, 44/76Mu-2, 44/76Mu-3, and 44/76Mu-4 (here designated Mu-1, Mu-2, Mu-3, and Mu-4, respectively), have been described previously (2, 9, 16). Strains 35E (NIBSC, Reference no. 2006) expressing LPS of immunotypes L2 and N4/96 were kind gifts from I. Feavers and E. Rosenqvist, respectively. The latter strain expresses an LPS type recognized by MAb, 223,D-8. This MAb binds to the LPS immunotype L1 reference strain 126E. Meningococci were grown on brain heart infusion (BHI) agar plates with 1% horse serum in a 5% CO2 atmosphere at 37°C. When the bacteria were grown in liquid culture, 800 ml of BHI broth with 1% horse serum in a 2-liter conical flask was inoculated with a bacterial suspension from two BHI plates and incubated at 37°C and 150 rpm overnight. All cultures were checked for purity by Gram staining.

**Preparation of NOMV and DOMV.** NOMV were isolated from overnight liquid cultures. The bacterial cells were removed by centrifugation, and the supernatant was concentrated 10-fold by ultrafiltration (500-kDa cutoff; A/G Technology Corporation, Needham, Mass.). The concentrate was then ultracentrifuged (100,000 g for 4 h) twice in order to remove medium components. The pellet was resuspended in deionized water and stored at −20°C. The NOMV preparations were tested for sterility prior to use. DOMV were prepared as previously described (1).

**Purification of LPS.** LPS was purified by hot water-phenol extraction (45), followed by ultracentrifugation (100,000 × g for 4 h) twice. The LPS pellet was freeze-dried and resuspended in 2% sodium deoxycholate (DOC)–2 mM EDTA. The LPS solution was then subjected to gel filtration chromatography (Sephacryl S-300; 2.5- by 95-cm column). The running buffer was 1% DOC and 2 mM EDTA in 20 mM Tris-HCl, pH 8.5. The eluate was monitored by refractive index and absorbance. Fractions giving signals by refractive index but not by UV absorbance at 250 or 280 nm were collected. The LPS was precipitated using cold ethanol-salt (40) and resuspended in deionized water. The cold ethanol-salt procedure was then repeated, and the precipitate was resuspended in deionized water and stored at −20°C.

**Quantification and dephosphorylation of LPS.** LPS was quantified by using a modified version of the method of Lyngby et al. (23a). Briefly, LPS samples were hydrolyzed in the presence of 2 M trifluoroacetic acid at 100°C overnight. Under these conditions, complete release of the 3-hydroxydecanoic acid from the lipid A portion of the LPS occurs. A known amount of 3-hydroxytridecanoic acid was added to the LPS sample before hydrolysis (45). After cooling, the 3-hydroxy fatty acids were recovered from the sample by solid-phase extraction (using Oasis HLB reversed-phase SPE cartridges containing 10 mg of sorbent [Waters Ltd., Watford, United Kingdom]) and reacted with the UV chromophore 2-bromoacetophenone to give phenacyl esters which could be detected by UV absorption at 240 nm. These were analyzed by reversed-phase high-pressure liquid chromatography, and by reference to a standard curve the

**Materials and Methods**

**Mice.** Female C57BL/6 mice (6 to 8 weeks old) were obtained from Charles River or Harlan Laboratories. Mice were maintained under specific-pathogen-free conditions. Experiments on animals were carried out according to regulations under the United Kingdom Home Office Animals (Scientific Procedures) Act of 1986.
amount of 3-hydroxydodecanoic acid in the original LPS sample could be calculated. LPS was completely dephosphorylated by treatment with aqueous hydroxide fluoride acid (48%, w/v) at 4°C for 48 h as previously described (6, 39).

**ELISA.** Microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with (per well) 100 μl of bovine serum albumin (BSA)-LPS complexes (0.125 μg of LPS/ml) dissolved in 0.05 M carbonate-bicarbonate buffer, pH 9.6. BSA-LPS complexes were prepared as previously described (5). Unoccupied protein binding sites were blocked with BSA (0.5%) in phosphate-buffered saline (PBS) for 1 h. Primary antibodies were incubated overnight at 4°C, and alkaline phosphatase-conjugated rabbit antibodies to mouse total Ig (1:1000; Southern Biotechnology Associates Inc., Birmingham, Ala.) were incubated for 2 h at room temperature. Between each incubation step the plates were washed five times with PBS containing 0.02% Tween 20. The substrate (p-nitrophenylphosphate, 1 mg/ml; Sigma, St. Louis, Mo.) was added in a 10% diethanolamine buffer, pH 9.8. The enzymatic reaction was terminated after 30 min by the addition of 50 μl of 1 M NaOH per well. The optical density (OD) was measured at 405 nm (Spectra MAX 340 enzyme-linked immunosorbent assay [ELISA] plate reader; Molecular Devices Corporation, Sunnyvale, Calif.). Results are expressed as reciprocal end point titer. A cutoff for positive OD values was calculated as the average plus three standard deviations of all dilutions from nonimmunized control mice (n = 3). The endpoint titer was defined as the reciprocal value of the first dilution below the cutoff.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.** Whole-cell preparations were made from overnight cultures (37°C) of bacterial cells on BH agar plates, suspended in 0.9% NaCl containing 0.02% sodium azide, inactivated (1 h at 56°C), and diluted to an OD of 0.2 at 600 nm. The whole-cell preparations were centrifuged, and the supernatants were removed and replaced by an equal volume of a sample buffer (23). Purified LPS suspended in deionized water was mixed 1:1 with sample buffer. All samples were boiled for 5 min before being applied to a 15% gel (23). LPS was visualized by silver staining (41). Electrophoretic transfer of proteins and LPS from the gel to nitrocellulose membranes was performed at 15 V for 15 min using a semidyed transblot cell (Bio-Rad Laboratories Inc., Richmond, Calif.). Unoccupied protein binding sites were blocked with 3% BSA in PBS for 1 h. Sera, MAbs, and peroxidase- or alkaline phosphatase-conjugated rabbit antibodies to mouse total Ig were incubated with BSA-LPS complexes (0.125 μg of LPS/ml) dissolved in 0.05 M phosphate-buffered saline (PBS) for 1 h. The enzymatic reaction was terminated after 30 min by the addition of 50 μl of 1 M NaOH per well. The optical density (OD) was measured at 405 nm (Spectra MAX 340 enzyme-linked immunosorbent assay [ELISA] plate reader; Molecular Devices Corporation, Sunnyvale, Calif.). Results are expressed as reciprocal end point titer. A cutoff for positive OD values was calculated as the average plus three standard deviations of all dilutions from nonimmunized control mice (n = 3). The endpoint titer was defined as the reciprocal value of the first dilution below the cutoff.

**Characterization of LPS in NOMV and DOMV from Mu-4.** LPS in NOMV and DOMV derived from Mu-4 was analyzed by SDS-PAGE and immunoblotting. SDS-PAGE with silver staining indicated that both the NOMV and DOMV preparations contained only one LPS band whose mobility was higher than that of the major LPS band of wild-type strain 44/76 (data not shown). Immunoblotting revealed that MAb 9-2-L379 (Fig. 2) (2, 4, 30), which is specific for an LPS epitope associated with the fully extended LPS carbohydrate chain, did not bind to LPS in the NOMV even when as much as 3.12 μg of protein was applied to the gel (Fig. 2). In contrast, MAB 216-Lc (4), which is specific for an inner core epitope, reacted strongly in immunoblots when as little as 0.375 μg of NOMV had been applied to the gel (Fig. 2). Furthermore, 1 ng of wild-type LPS was easily detected by MAB 9-2-L379 in immunoblots. Consequently, if the OMV preparations used in our study contained any wild-type LPS of immunotype L3,7, it would be in amounts of less than 0.32 ng/μg of protein. The NOMV and DOMV preparations were also devoid of immunotype L8 structures as determined by using MAbs 2-1-L8 in similar immunoblotting analyses.

**Quantification of serum antibodies to Mu-4 and 44/76 LPS by ELISA.** Sera from mice that were immunized i.v., i.p., or i.n. with NOMV and DOMV from Mu-4 were tested by ELISA for antibodies to LPSs from Mu-4 and wild-type strain 44/76. End point titers for individual sera are shown in Fig. 3. Although there was considerable mouse-to-mouse variation within each group, altogether 20 of 26 samples collected at various time points from 20 mice after immunization contained high levels of antibodies to Mu-4 LPS (Fig. 3). Interestingly, 8 of the 26 sera tested also had high titers of antibodies to LPS from strain 44/76, ranging from 1:1,250 to 1:80,000. The LPS from strain 44/76 is primarily of immunotype L3,7 (Fig. 3).

The highest frequency of sera with antibodies to 44/76 LPS was found in the i.v.-immunized group. The antibody titers to
wild-type LPS were similar to, or significantly lower than, titers to Mu-4 LPS. Moreover, antibodies to the 44/76 LPS were not detected in the absence of antibodies to Mu-4 LPS, but the reverse was frequently observed. Similar results were obtained in two other experiments using serum pools collected at day 11 or 12 and at day 30 or 32 after the last dose of i.n. immunization with NOMV or DOMV (data not shown). Generally the levels of antibody levels to LPS were lower at day 30 or 32 than at day 11 or 12 after the last immunization. These observations together suggest that the Mu-4 LPS incorporated in NOMV or DOMV induced at least two populations of antibodies to the inner core with different specificities: one specific for the non-substituted inner core structure and one specific for the inner core when it is substituted with additional sugar residues.

**Determination of the specificity of antibodies to LPS by immunoblotting.** Immunoblotting was used to analyze the specificity of polyclonal antibodies to wild-type LPS from strain 44/76 in serum pools from mice immunized i.n. with NOMV or DOMV (Fig 4a) and in individual sera from mice immunized i.n., i.p., or i.v. (Fig. 4b). LPS from strain 44/76 is heterogeneous and separates into two bands on SDS-PAGE (Fig. 4a). The major band, with lower mobility, contains LPS with the fully extended carbohydrate chain of immunotypes L3 and L7 recognized by MAb 9-2-L379 and other MAbs with similar specificity (2). The minor band, with higher mobility, contains LPS molecules with shorter oligosaccharide chains of immunotype L8 (data not shown) (Fig. 1) and LPS having only the complete inner core, similar to Mu-4. The latter LPS type is recognized by MAb 216-Lc (Fig. 1 and 4a).

Mouse sera that contained high levels (end point titer, >1:40,000) of antibodies to 44/76 LPS as determined by ELISA stained both bands of 44/76 LPS in immunoblotting (Fig. 4b). The lower band is stained more strongly than the upper band. In contrast, sera that showed detectable antibodies only to Mu-4 LPS in ELISA failed to stain the upper LPS band but stained the lower LPS band strongly (Fig. 4b). Immunoblotting with MAb 216-Lc confirmed that the upper SDS-PAGE band of 44/76 LPS did not contain the Mu-4 LPS phenotype (Fig. 4a). Thus, the observed binding to both bands of 44/76 LPS by sera from mice immunized with Mu-4-derived NOMV or DOMV demonstrates that anti-Mu-4 LPS antibodies can also bind to wild-type LPS having the fully extended oligosaccharide chain. Serum from a rabbit immunized with NOMV from Mu-4 was also found to contain similar cross-reactive anti-LPS antibodies. The binding to wild-type LPS was completely inhibited by 0.227 mM free Mu-4 LPS (data not shown). Similar studies could not be done with mouse sera due to insufficient quantities remaining after ELISA and immunoblotting analyses.

Immunoblotting also revealed that the cross-reactive epitopes are absent in LPSs from strains N4/96 and 35E (data not shown). LPS from strain N4/96 is recognized by MAb 223,D-8 which binds to the immunotype L1 reference strain 126E. Strain 35E is the reference strain for immunotype L2. Whole-cell preparations from neither of the strains contained LPS structures recognized by MAb 9-2-L379, 2-1-L8, or 216-Lc (data not shown).

In order to delineate the cross-reactive epitope recognized by these polyclonal antibodies, sera were tested for binding to LPSs from three other inner core mutants: Mu-1, Mu-2, and Mu-3 (see Fig. 1 for structures). Some sera stained Mu-3 LPS but did so significantly more weakly than with Mu-4 LPS (data not shown). None of the sera bound to Mu-2 or Mu-1 LPS (data not shown). This indicates that the internal epitope responsible for the induction of antibodies cross-reactive to wild-

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**FIG. 3.** Antibodies to LPS from Mu-4 (●) and the wild-type strain 44/76 (○) in sera from mice immunized with NOMV derived from Mu-4. The results for individual mice are expressed as log_{2} of the end point titer. A cutoff was calculated as the average plus three standard deviations of all dilutions from nonimmunized control mice (n = 3). The highest starting dilution of sera was 1:1,250. *, days after booster. Neg.Ctr., negative control.
type LPS is expressed mainly by the Mu-4 LPS structure comprising the complete inner core (Fig. 1).

Sera were further tested by immunoblotting for binding to dephosphorylated wild-type LPS. Dephosphorylation of LPS abolished the antibody binding in all sera except one serum pool (Fig. 5). These results show that cross-reactive anti-LPS antibodies can be divided into at least two distinct specificities: one that is dependent on phosphoethanolamine groups for binding and one that is not. Total dephosphorylation also abolished the binding of MAb 9-2-L379 but not the binding of MAb 216-Lc.

**DISCUSSION**

There is evidence supporting the view that antibodies to LPS could be protective against meningococcal infections in humans. However, their effectiveness would most likely be dependent on their antigen affinity, epitope specificity, and isotype. Due to the inherent heterogeneity and rapid phase variation of meningococcal LPS, protective antibodies to LPS should ideally be able to bind to variants of all 11 immunotypes.

This study describes the antibody response to LPS in NOMV and DOMV derived from Mu-4 of *N. meningitidis* strain 44/76. This LPS mutant synthesizes LPS with a truncated carbohydrate chain consisting of only the complete inner core (2). The NOMV and DOMV preparations used for immunization were shown by immunoblotting to be devoid of wild-type LPS of immunotype L3,7 or to contain LPS components with SDS-PAGE mobility similar to that of the L3,7 LPS band. Consequently, antibodies induced by the LPS in these preparations could be directed only towards epitopes in the inner core structure of LPS.

A significant number of polyclonal sera from mice immunized by all three routes were found to contain high levels of antibodies to Mu-4 LPS by both ELISA and immunoblotting. Some of these immune sera also reacted strongly to wild-type LPS, which consists predominantly of immunotype L3,7 LPS. These results indicate that LPS carrying only the inner core of the carbohydrate chain can induce at least two distinct popu-
lations of antibodies: one population that binds to nonsubstituted inner core oligosaccharides and another that binds to the inner core when it is substituted with oligosaccharides that constitute the immunotype L3,7.

In previous studies, polyclonal sera and the MAb 216-Lc raised against meningococcal LPS with truncated oligosaccharide chains (immunotype L8a and Mu-4 LPS, respectively) were also found to bind to purified LPS and whole bacteria from the wild-type strain 44/76 in ELISA (4, 15). MAb 216-Lc also reacted with moderate strength to a wide range of meningococcal case strains (4). Immunoblotting, however, revealed that both the polyclonal antibodies and MAb 216-Lc bound only to the minor LPS band from 44/76, which contains LPS variants with truncated oligosaccharide chains homologous to the LPS used for immunization. No binding to the band containing LPS with the fully extended carbohydrate chain of immunotype L3,7 was apparent (4, 15).

This discrepancy could be due to the fact that some antibodies to the inner core bound to the fully extended wild-type LPS with low avidity in one assay but did not do so in another assay. However, it is more likely that the cross-reactivity observed in ELISA could be due to antibodies that bound to the minor populations of LPS molecules having only the truncated carbohydrate chain. Serial dilutions of MAb 216-Lc in ELISA plates coated with whole cells from the wild-type strain showed that the moderate binding was due to a limiting amount of antigen rather than low-avidity binding to the fully extended LPS in ELISA (S. R. Andersen, unpublished data). Consequently, the antigenic heterogeneity within LPS preparations used as immunogen or antigen can easily occlude the true specificity of anti-LPS antibodies. Therefore, methods such as SDS-PAGE and immunoblotting should be included in the characterization of antibodies to LPS.

The observed cross-reactivity of antibodies to the heterogeneous wild-type LPS in ELISA in the present study also could have been a result of antigenic heterogeneity in the wild-type LPS preparation. However, immunoblot analysis clearly showed that some sera contained antibodies that were able to bind not only to the Mu-4 LPS but also to the LPS band comprising fully extended LPS of immunotype L3,7. Immunoblot analysis with MAB 216-Lc showed that the L3,7 LPS band of 44/76 LPS did not contain LPS structures similar to that of Mu-4.

The binding to the upper LPS band is weaker than that to the lower band. This could be due to the fact that the cross-reactive antibodies bind to the fully extended LPS with reduced avidity compared to the binding to the LPS in the lower band. Alternatively, the observed difference in staining intensity could be caused by quantitative differences between cross-reactive antibodies and antibodies binding exclusively to the lower band. Unfortunately, we were unable to separate LPS containing the L8 and the Mu-4 inner core by SDS-PAGE and thus cannot confirm whether or not the cross-reactive anti-LPS antibodies bind LPS of immunotype L8.

The polyclonal sera in the present study did not cross-react to LPSs from two meningococcal strains, N4/98 and 35E, which are representative of L1 and L2 immunotypes, respectively. The lack of binding to N4/96, which has LPS similar to immunotype L1 LPS, is slightly surprising, as the inner core structure of L1 is identical to that of L3,7 (11, 22, 33). The only known structural difference between L3,7, and L1 is that L1 has a digalactose moiety whereas L3,7 has a sialylated or nonsialylated lacto-N-neotetraose moiety attached to heptose I of the inner core. The lack of cross-reactivity between immunotypes L3,7 and L1 could thus be due to differences in the exposure of inner core epitopes caused by structural differences in the outer core region. Alternatively, there are strain-specific structural differences, e.g., in phosphorylation pattern, between the well-characterized L1 structure produced by 35E and LPS from N4/96. Further characterization of the N4/96 LPS is necessary in order to clarify this issue. The lack of reaction to L2 is less surprising, as the L2 inner core has an additional glucose residue attached to position 3 and a phosphoethanolamine group attached to position 6/7 on heptose II (13).

The structural motifs that express the epitopes recognized by the cross-reactive antibodies in this study seem to include the entire inner core structure of Mu-4 LPS. A minority of the sera bound weakly to LPS from Mu-3, which lacks the terminal glucose attached to heptose I. None of the sera bound to LPS

FIG. 5. SDS-PAGE and immunoblotting of native and dephosphorylated LPS from strain 44/76 with serum pools from mice immunized i.n. or i.v. with NOMV or DOMV from Mu-4. The arrows on the left indicate the two LPS bands of 44/76 LPS. One microgram of native LPS and 5 μg of dephosphorylated (de-Phos.) LPS were applied.
from Mu-2, which lacks both the terminal glucose and the GlcNAc attached to heptose II, or to Mu-1 LPS, whose carbohydrate portion consists only of two keto-deoxyoctulosonic acid (KDO) residues (Fig. 1). Similar binding patterns were found for MAbs B5 and 216-Lc (4, 34). Also, purified Mu-4 LPS was able to completely block the binding of antibodies to both LPS bands of the wild-type LPS in immunoblotting (S. R. Andersen, unpublished data).

The two MAbs B5 and 216-Lc differ in their requirement for the presence of the phosphoethanolamine group at position 3 in heptose II. MAb B5 does not bind if the phosphoethanolamine group is located at position 6/7 or is absent, whereas 216-Lc binds equally well to native and dephosphorylated LPS (4, 34). All but one serum pool in the present study required the presence of phosphoethanolamine residues, and the other is not.

The presence of phosphoethanolamine groups in the inner core may thus be important, but is not crucial, for the induction of cross-reactive antibodies.

The cross-reactive antibodies could potentially have been induced by LPS structures in the normal flora homologous to the LPS of immunotype L3,7 since high levels of antibodies to meningococcal LPS have previously been found in premunine sera from rabbits (43). However, the cross-reactive antibodies described in this study are more likely to be induced by immunization with NOMV and DOMV containing Mu-4 LPS for the following reasons. First, antibodies to the wild-type LPS were only found in sera from mice which also had high levels of antibodies to Mu-4 LPS. Second, the levels of both Mu-4 and 44/76 LPS antibodies dropped relatively soon after the first round of immunizations. If antibodies were induced by normal flora, they would probably still be present at a later time point.

Third, nonimmunized mice kept under the same conditions did not contain detectable antibodies to Mu-4 or wild-type LPS.

Experiments are now in progress to isolate truly cross-reactive MAbs with specificities similar to those found among polyclonal antibodies. Such MAbs will be important tools for defining inner core epitopes that are still accessible on the wild-type LPS with a fully extended carbohydrate chain. Furthermore, MAbs will also be useful to evaluate the potential protective capacity of cross-reactive antibodies to LPS in functional assays and animal models. The main conclusion of this study is that LPS with a complete inner core incorporated in NOMV or DOMV vaccines can induce antibodies which can cross-react between LPS variants from the same immunotype with different carbohydrate chain lengths. Mu-4 LPS or oligosaccharides derived from Mu-4 LPS could be important antigens to include in future vaccines against meningococcal disease. Such cross-reactive antibodies could potentially prevent bacteria from escaping antibody-mediated killing by LPS phase variation, but this will need to be investigated.

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