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A New Serogroup (L) of *Neisseria meningitidis*

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A strain of *Neisseria meningitidis* (LCDC 78189) isolated from the mother of a 3-year-old male with meningococcal meningitis was found to be antigenically distinct from the known serogroups A, B, C, D, H, I, K, X, Y, Z, 29E, and W135; it was designated serogroup L. Anti-78189 serum specifically agglutinated the homologous strain and three other strains which were isolated from the father and two other contacts of the child. Only those strains isolated from the contacts produced immunoprecipitates with the anti-78189 serum by the antiserum-agar method. A structurally unique capsular polysaccharide which was obtained from strain 78189 in a highly purified state was demonstrated to be the antigen responsible for the serological properties of the strain. The polysaccharide formed a precipitin band with the anti-78189 serum but not with the meningococcal grouping sera, and it was also able to absorb both the agglutinating and precipitating activity from the anti-78189 serum.

Serogrouping of *Neisseria meningitidis* is based upon the recognition by antibody of antigenically distinct capsular polysaccharides. In 1954, the Subcommittee on the Family Neisseriaceae recommended that *N. meningitidis* be classified into serological groups A, B, C, and D (30) in accordance with the findings of Branham (6). In 1961, Slaterus (28) described three new serogroups (X, Y, and Z) and later described a fourth serogroup (Z') which was related to serogroup Z (29). In 1968, Evans et al. (12) proposed three more serogroups which were identified as Bo, 29E, and 135 (later termed W135). It is now known that Bo and an E group described by Vedros et al. (31) correspond to Slaterus group Y, and Z' corresponds to 29E (4, 12, 13).

Presently, A, B, C, D, X, Y, Z, 29E, and W135 are recognized as classical serogroups. With the exception of group D, the capsular polysaccharides of these serogroups have been isolated and characterized chemically and structurally (3–5, 8, 18, 19, 21, 22). Three antigenically distinct serogroups, H, I, and K, were described recently (11); however, the capsular polysaccharides of these strains have yet to be isolated and characterized.

This communication describes the identification of a new serogroup of *N. meningitidis* based on serological characterization of the strain. This evidence is also supported by the isolation from the strain of a unique capsular polysaccharide which has been demonstrated to be the major antigen responsible for the serological properties of the strain. Structural studies on the capsular polysaccharide are described elsewhere (H. J. Jennings, C. Lugowski, F. E. Ashton, and A. Ryan, Carbohydr. Res., in press).

MATERIALS AND METHODS

Bacteria. Meningococcal prototype strains (serogroups shown in parentheses) used in this study were 604 (A), 608 (B), 2241 (C), M623 (D), M405 (X), Slaterus Y (Y), Slaterus Z (Z), 614 (W135), and M550 (29E). Strains M623, M405, Slaterus Y, Slaterus Z, and M550 were obtained from N. A. Vedros, University of California, Berkeley. Strains 604, 608, 2241, and 614 were from the culture collection of the National Reference Centre for Neisseria, Ottawa, Ontario, Canada. Strains 1890 (H), 1486 (I), and 1811 (K) were supplied by D. Shaoquing, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Four strains of *N. meningitidis* (L.C.D.C. 78187, 78188, 78189 and 78190) isolated from the throats or noses of the parents and two other adults who were contacts of a 3-year-old male with meningococcal meningitis were received from the Provincial Laboratory of Public Health, Ottawa, Ontario, Canada, for confirmation and identification of the serogroup. The strain which infected the child was not available for characterization. Confirmation of strains as *N. meningitidis* was carried out with cysteine-Trypticase (BBL Microbiology Systems, Cockeysville, Md.) agar (32) and starch gelatin medium (Institut Armand Frappier, Laval-des-Rapides, Quebec, Canada).

Serogrouping. *N. meningitidis* strains grown on either Columbia blood agar (BBL Microbiology Systems) or GC medium (20) were serogrouped by bacterial slide agglutination (2) and the antiserum-agar method (1, 2, 10). Rabbit antisera were prepared as described previously (2). Group A, B, and C horse

sera used in the antiserum-agar method were obtained from C. E. Frasch, Food and Drug Administration, Bethesda, Md.

Agar gel double diffusion. Immunodiffusion was performed in accordance with the method of Ouchterlony (25) with 1% agarose Indubiose A37 (Accurate Chemical and Scientific Corp., Hicksville, N.Y.).

Wheat germ agglutination. The procedure was that of Schaefer et al. (27); wheat germ (62 μg/ml) from *Triticum vulgaris* (Sigma Chemical Co., St. Louis, Mo.) was used. Encapsulated and nonencapsulated strains were used as negative and positive controls, respectively.

Capsular polysaccharide. Polysaccharide was isolated and purified from strain 78189 as described previously (7).

Absorption of antiserum. The anti-78189 rabbit serum was incubated with its soluble homologous capsular polysaccharide at the equivalence point (100 μg of polysaccharide per 0.1 ml of antiserum) at 37°C for 1 h. A control experiment, in which the same antiserum without the addition of polysaccharide was used, was also carried out. The incubated antisera were centrifuged at 6,000 × *g* for 15 min, and the supernatants were used for agglutination and antiserum-agar experiments.

Analytical methods. Analyses for amino sugar were performed on a Technicon autoanalyzer. Acid hydrolysis of the polysaccharide was carried out with 4 M HCl at 100°C. Amino sugar determinations were carried out at intervals between 4 and 24 h after incubation of the hydrolysates with alkaline phosphatase (Worthington Biochemical Corp., Freehold, N.J.). The total 2-amino-2-deoxy-glucose content was determined by extrapolation to zero time. Phosphorus was determined by the method of Chen et al. (9). Protein was determined by the method of Lowry et al. (23), and lipopolysaccharide was determined by estimation of 2-keto-3-deoxyoctonate by the method of Weisbach and Hurwitz (33). Nucleic acid was determined by UV absorption at 260 nm. The ^{13}C nuclear magnetic resonance (NMR) spectrum of the polysaccharide was recorded at 20 MHz in the pulsed Fourier transform mode with complete proton decoupling. Chemical shifts were reported in parts per million downfield from external tetramethylsilane, and the ^{2}H resonance of deuterium oxide was used as the field-frequency lock signal. The polysaccharide was run as a deuterium oxide solution (50 mg/ml).

RESULTS

Identification and characterization of meningococci. The meningococcal strains 78187, 78188, 78189, and 78190 exhibited colony morphology and growth typical of *N. meningitidis*. The strains produced acid from glucose and maltose but not from sucrose, mannitol, fructose, and lactose. The organisms were gram-negative diplococci and were oxidase and catalase positive. The strains failed to grow on chocolate or Columbia blood agar at 22°C and did not cause hemolysis on 5% sheep blood agar. The strains did not synthesize polysaccharide from 5% sucrose (26) and failed to agglutinate in wheat germ.

Serology. Antigens prepared from confluent growth of strains 78187, 78188, 78189, and 78190 tended to autoagglutinate slightly in saline and normal rabbit serum. This problem was essentially overcome by preparing antigens from several single colonies rather than from confluent areas of growth. The four strains were nonagglutinable in the prototype meningococcal grouping sera, including the anti-H, -I, and -K sera. An antiserum made to strain 78189 agglutinated the homologous strain and the three related strains 78187, 78188, and 78190.

When tested with the antiserum-agar method, the anti-78189 serum formed immunoprecipitates with the homologous strain and the three related strains 78187, 78188, and 78190 but not with the prototype strains (Fig. 1). Conversely, none of the prototype sera, including the anti-H, -I, and -K sera, produced immunoprecipitates with the four strains (data not shown). Absorption of the anti-78189 serum with the capsular polysaccharide from strain 78189 removed its agglutinating activity and its ability to form an immunoprecipitate when tested with the antiserum-agar method.

Figure 2 shows the results of agar gel double diffusion when the anti-78189 serum and other grouping sera were reacted against purified capsular polysaccharide from *N. meningitidis* strain 78189. Only the anti-78189 serum formed a precipitin band with the purified polysaccharide.

Characterization of the capsular polysaccharide. ^{13}C NMR spectroscopic analysis provides a convenient fingerprint for the recognition of each of the meningococcal polysaccharides. The ^{13} NMR spectrum of the newly isolated polysaccharide is shown in Fig. 3.; it is different from the ^{13}C NMR spectra of all the known meningococcal serogroups (3–5, 8, 19). A full analysis of the ^{13}C NMR spectrum of the polysaccharide in relation to its structure has been reported elsewhere (H. J. Jennings, C. Lugowski, F. E. Ashton, and A. Ryan, Carbohydr. Res., in press); this analysis indicated that only signals associated with carbon atoms of the polysaccharide structure could be detected in the spectrum. The chemical analysis of the polysaccharide is shown in Table 1. The polysaccharide contained only glucosamine and phosphate in the molar ratio of 3:1. Protein, nucleic acid, and lipopolysaccharide were only detected in extremely small quantities.

DISCUSSION

Meningococcal strains 78187, 78188, 78189, and 78190 were isolated from contacts of a 3-year-old male with meningococcal meningitis. Unfortunately, the strain which infected the child was not available for study, but strain 78189 was selected for the preparation of antise-

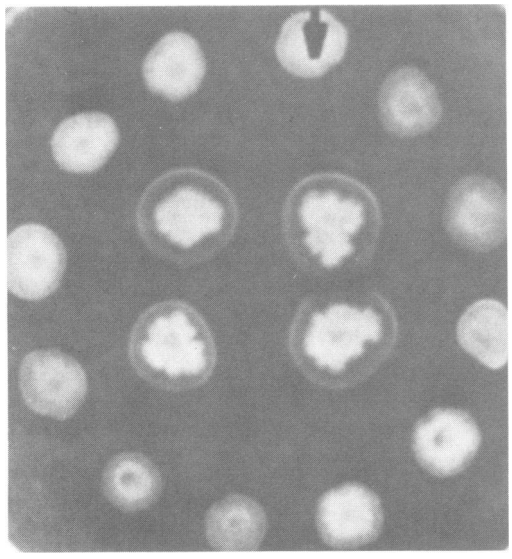

FIG. 1. Antiserum-agar method results for anti-78189 serum. Agar contained serum at a final dilution of 1:5. Shown are (proceeding in a clockwise direction around the periphery of the agar, starting at the arrow) the following strains (serogroups in parentheses): 604 (A), 608 (B), 2241 (C), M623 (D), M405 (X), Slaterus Y (Y), Slaterus Z (Z), M550 (29E), 1890 (H), 1486 (I), and 1811 (K). The four strains which are centrally located and are surrounded by immunoprecipitates were isolated from four different contacts, including the mother and father, of the child with meningitis.

ra and capsular polysaccharide because it was isolated from the mother of the child. It is unknown whether the strain isolated from the mother caused the disease in the child, but other studies have indicated that mothers are most likely to be carriers of the disease strain within nuclear families (14, 15). The father of the child also carried the same strain (78190) as did the mother, and it has been shown that meningococcal infection is usually introduced into families by adults, with disease eventually occurring in the child (24).

The initial recognition of encapsulation of these newly isolated meningococcal strains was based on their failure to agglutinate in wheat germ. This test is based on the ability of the capsule to impede the access of lectin to *N*-acetylglucosamine residues (16) associated with the subcapsular meningococcal lipopolysaccharides.

The major specificity of wheat germ agglutinin is for internal β-D (1 → 4)-linked *N*-acetylglucosamine residues, and the presence of these residues has recently been demonstrated in the oligosaccharide units of the R-type meningococcal lipopolysaccharides (H. J. Jennings, unpublished data). The optimal binding to wheat germ agglutinin is given by β-D (1 → 4)-linked *N*,*N*′,*N*″-triacetylglucosamine (chitotriose) (17), and it is interesting to note that the repeating unit of the group L polysaccharide (Fig. 3) also contains a similar β-D-linked *N*,*N*′,*N*″-triacetylglucosamine unit. However, fortuitously, in this case the trisaccharide contained only 1 → 3 linkages, obviously rendering it unable to bind to wheat germ agglutinin.

Serological studies show that strains 78187, 78188, 78189, and 78190 are identical to each other and antigenically distinct from the other known meningococcal serogroups, including the recently defined H, I, and K serogroups (11). All the strains were nonagglutinable in grouping sera, and of all the meningococcal serogroups, only the above strains formed immunopre-

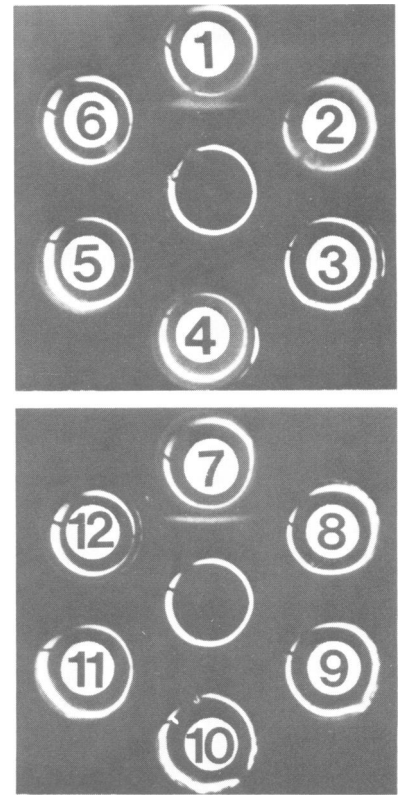

FIG. 2. Double diffusion in agar of meningococcal grouping sera against purified capsular polysaccharide from *N. meningitidis* 78189. Central wells contain polysaccharide. Sera were as follows (serogroups in parentheses): 1, anti-78189; 2, anti-604 (A); 3, anti-608 (B); 4, anti-2241 (C); 5, anti-Slaterus Y (Y); 6, anti-Slaterus Z (Z); 7, anti-78189; 8, anti-M550 (29E); 9, anti-614 (W135); 10, anti-1890 (H); 11, anti-1486 (I); and 12, anti-1811 (K). Not shown are anti-M405 (serogroup X), anti-M623 (serogroup D), and preimmune (78189) sera, which did not react with the purified capsular polysaccharide. The photograph was taken 24 h after the commencement of diffusion.

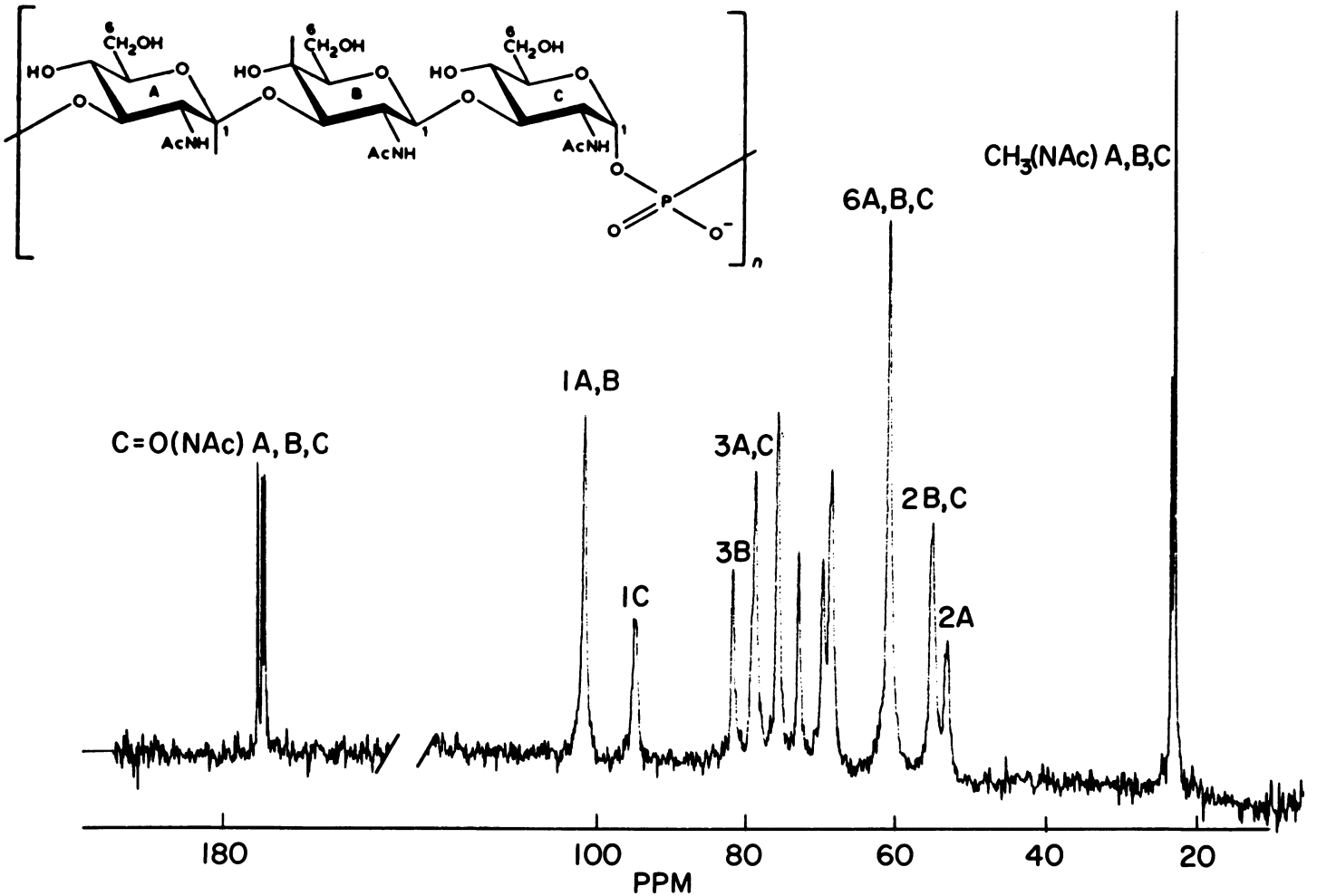

FIG. 3. Fourier-transformed ^{13}C NMR spectrum of the polysaccharide obtained from *N. meningitidis* 78189 taken with an acquisition time of 0.5 s and a spectral width of 5 KHz. The number of free-induction decays was 120,000.

TABLE 1. Analysis of polysaccharide from *N. meningitidis* 78189

Component	% by wt[a]	Molar ratio
Glucosamine	76.1	2.91
Phosphorus	4.4	0.94
2-Keto-3-deoxyoctonate	<0.1	
Protein	<1	
Nucleic acid	<1	

[a] Corrected for moisture.

cipitates with the anti-78189 serum when tested with the antiserum-agar method. That the principal antigen involved in the above serological experiments was the capsular polysaccharide was established when a preparation of the polysaccharide from strain 78189 was able to remove all the agglutinating activity and the ability to form immunoprecipitates with the homologous strain from the anti-78189 serum. The capsular polysaccharide was also demonstrated to be group specific in immunodiffusion experiments.

An important factor in the interpretation of the above serological results is the extent of the purity of the polysaccharide preparation. This was initially determined by ^{13}C NMR spectroscopy when only signals associated with the carbon atoms of the polysaccharide were detected (Fig. 3). A more accurate assessment of the purity of the capsular polysaccharide could be obtained by chemical analysis (Table 1). Glucosamine and phosphate were the only components detected in any significant quantities and accounted for 80.5% of the total weight of the polysaccharide. Most of the remainder could be attributed to *N*-acetyl groups (calculated as 18.5%). The fact that all three of the glucosamine residues of the repeating unit of the polysaccharide were *N*-acetylated could be determined from three signals of equal intensity at approximately 176 ppm in the ^{13}C NMR spectrum of the polysaccharide (Fig. 3). These signals are characteristic of the carbonyl signals of the acetamido group (8, 18).

The above evidence indicates that strain 78189 represents a new meningococcal serogroup, based on its serological properties and the isolation of a unique capsular polysaccharide responsible for these serological properties; it has been designated group L.

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