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Role of Lipopolysaccharide in Wheat Germ Agglutinin-Mediated Agglutination of *Neisseria meningitidis* and *Neisseria gonorrhoeae*

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Wheat germ agglutinin, having specificity for *N*-acetyl glucosamine, agglutinated known nonencapsulated *Neisseria meningitidis* strains, but failed to agglutinate encapsulated strains of all serogroups tested. Presence of a capsule, therefore, blocked wheat germ agglutinin agglutination of *N. meningitidis* strains. In contrast, *Neisseria gonorrhoeae* strains were strongly agglutinated, providing additional evidence for nonencapsulation of *N. gonorrhoeae*. Purified lipopolysaccharide from a single *N. meningitidis* strain specifically blocked wheat germ agglutinin agglutination of all *N. meningitidis* and *N. gonorrhoeae* strains tested. Thus, in absence of capsular polysaccharide wheat germ agglutinin agglutinates *Neisseria* strains through interaction with lipopolysaccharide in the outer membrane. Of 34 nongroupable throat *N. meningitidis* isolates, 10 failed to agglutinate in wheat germ agglutinin, suggesting that at least some nongroupable *N. meningitidis* strains may possess capsule-like materials.

Approximately 50% of *Neisseria meningitidis* isolates recovered from carriers are not serogroupable (2, 6). The nongroupable strains have been divided into those that are saline-agglutinable, those that do not agglutinate in either saline or group-specific antisera, and those that agglutinate in multiple-grouping sera (4). When examined by the antiserum agar method for serogroup identification, strains representing each of these agglutination patterns remained nongroupable (3). Most of the nongroupable strains presumably lack a capsular polysaccharide.

It has not been possible to subdivide *Neisseria gonorrhoeae* into serogroups comparable to those of *N. meningitidis*. Although six antigenically different lipopolysaccharide (LPS)-derived acidic polysaccharides have been isolated from different strains of *N. gonorrhoeae* (1), there are no reports of true capsular polysaccharides from this organism.

Schaefer et al. (13) reported that wheat germ agglutinin (WGA) could specifically identify isolates of *N. gonorrhoeae* recovered from the genital-urinary tract. WGA is specific for *N*-acetyl glucosamine, and this compound specifically blocks the WGA-mediated agglutination of gonococcal strains (13). Both gonococcal and meningococcal LPS contain large amounts of *N*-acetyl glucosamine (8, 15). The present study shows that *N. meningitidis* and *N. gonorrhoeae* strains were agglutinated by WGA through binding of WGA to LPS and that the agglutination was blocked by the presence of a capsule. Nongroupable throat isolates of *N. meningitidis* were examined for agglutination in WGA and most, but not all, agglutinated and were, therefore, presumably nonencapsulated.

MATERIALS AND METHODS

Strains and growth conditions. Meningococcal strains used in these studies with M-prefixes were prototype strains (5), those with CL-prefixes were recovered from healthy carriers (2), and those with BB-prefixes were submitted to our laboratory for identification. The strains were grown overnight on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) containing 1% normal horse serum. Nonencapsulated variants were selected from parent strains as naturally occurring mutants by use of antiserum agar (3). The gonococcal control strains used were 2686 and two isolates received from the reference collection of King Holmes, Seattle, Wash.

Agglutination and inhibition of agglutination. The agglutination procedure was that of Schaefer et al. (13) using WGA from *Tritcum vulgaris* (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 62 μg/ml. *N*-acetyl glucosamine (Sigma) at 10 mg/ml in 0.05 M phosphate-buffered saline, (PBS; pH 7.4) was used to specifically inhibit the agglutination reaction. The quantitative inhibition test was performed in U-well microtitration plates using protein-free LPS, as determined by amino acid analysis. The LPS was prepared by detergent solubilization from outer membrane material from a known nonencapsulated strain (M986-NCV-1) and from strain M981 (7). The LPS was then dissolved in distilled water and diluted in twofold serial dilutions from 2,500 to 10 μg/ml. Twenty microliters of each LPS dilution was combined with

20 μl of 62-μg/ml WGA in PBS followed immediately with 20 μl of organisms in PBS. The plates were tapped gently to mix the contents and read with the aid of a dissecting microscope as soon as maximal agglutination had occurred in the controls (2 to 4 min). The concentration of LPS causing ca. 50% inhibition was determined.

Three WGA-agglutinable meningococcal strains were treated with *Clostridium perfringens* neuraminidase (Sigma). Approximately 5×10^9 organisms in 1 ml of 0.10 M sodium acetate buffer (pH 5.5) in 0.05 M NaCl were treated with 0.5 U of neuraminidase for 60 min at 37°C, pelleted, and suspended in 1 ml of PBS.

RESULTS

In a preliminary experiment to confirm that encapsulated strains of *N. meningitidis* were not agglutinated by WGA, 24 serogroupable isolates were examined (Table 1). None of the encapsulated organisms was agglutinated by the lectin.

The assumption was made that nonencapsulated *N. meningitidis* strains have *N*-acetyl glucosamine groups exposed on their surface and would, therefore, be agglutinated by the WGA. This was tested by comparing the reactivity of six encapsulated parent strains with their nonencapsulated variants (Table 2). All six of the nonencapsulated variants were strongly agglutinated by the WGA. Thus, presence of a capsular polysaccharide blocked WGA-mediated agglutination.

It has been assumed that most nongroupable *N. meningitidis* isolates are not serogroupable due to absence of a capsular polysaccharide. We, therefore, examined 34 randomly selected nongroupable throat isolates recovered from healthy carriers (2) for agglutinability by WGA (Table 1). A total of 17 strains were agglutinated by WGA, and an additional 7 strains, some of which appeared to aggregate to a greater extent in the presence of WGA, agglutinated in PBS. None of the six noncapsular variants were saline agglutinable (Table 2), yet 20% of the nongroupable throat isolates were saline agglutinable. Ten of the nongroupable strains (29%) failed to agglutinate in the WGA. These strains were reexamined by antiserum agar for reactivity with A, B, C, Y, W135, and 29E sera, and by slide agglutination for reactivity with X and Z sera. All 10 strains remained nongroupable. LPS was prepared from 8 of these 10 strains, examined for glucosamine content, and found to contain the expected amounts of glucosamine (data not shown).

TABLE 1. *Comparison of groupable and nongroupable meningococcal strains for agglutination by WGA*

Serogroup	No. of strains	WGA agglutinated (no. of strains)	PBS agglutinated[a] (no. of strains)
B	8	0	0
C	4	0	0
X	3	0	0
Y	3	0	0
W135	3	0	0
29E	3	0	0
Nongroupable	34	17	7
Gonococci	3	3	0

[a] Those strains which autoagglutinated in PBS were not counted among the strains that were agglutinated by WGA.

TABLE 2. *Reactivity of WGA with known nonencapsulated meningococcal strains*

Strain	Serogroup of parent strain	Agglutination of strain[a]			
		Parent		Variant	
		WGA	PBS	WGA	PBS
S-4185	A	−	−	3+	−
M136	B	−	−	3+	−
M981	B	−	−	4+	−
M986	B	−	−	4+	−
BB-119	B	−	−	4+	−
BB-92	Y	−	−	4+	−

[a] The extent of agglutination graded from − (negative) to 4+ (very strong).

The specificity of the WGA agglutination was examined for six of the WGA-positive strains. The only meningococcal cell surface component known to contain glucosamine is the LPS (12). All meningococcal LPSs thus far examined contain 10 to 15% glucosamine by weight (C. M. Tsai and C. E. Frasch, unpublished data). Highly purified LPS from two different *N. meningitidis* strains at 5 mg/ml completely blocked WGA-mediated agglutination of both meningococcal and gonococcal strains. As expected, 10 mg of *N*-acetyl glucosamine per ml also blocked the agglutination. The minimum amount of LPS required for 50% inhibition of agglutination was determined (Table 3). A mean of 23 μg of LPS per ml was required for 50% inhibition of the six meningococcal strains. The meningococcal LPS at a concentration of 78 μg/ml also blocked agglutination of gonococcal strain 2686. When a similar titration was done with *N*-acetyl glucosamine, more of the sugar was required for equivalent inhibition. Neuraminidase treatment did not abolish the agglutination of these meningococcal strains by WGA, thus providing additional evidence for the specificity of the observed agglutination.

TABLE 3. *Inhibition of WGA agglutination of meningococcal strains by M986 LPS*

Strain	Agglutination without inhibitor[a]	Amt of LPS required for 50% inhibition (μg)
M981-NCV-1	2+	10
M136 NCV-3	4+	10
CL-32	4+	20
C1-318	3+	20
CL-321	4+	39
CL-328	2+	39
2686[b]	4+	78

[a] The extent of agglutination graded from − (negative) to 4+ (very strong).

[b] Strain 2686 is a laboratory strain of *N. gonorrhoeae*.

DISCUSSION

The specific neisserial component through which WGA interacts to effect agglutination is most likely the LPS. Analysis of the outer membranes of various meningococcal strains indicates that the LPS is the only membrane component thus far found to contain glucosamine (14). There are eight reported LPS immunotypes in *N. meningitidis* (16) and six in *N. gonorrhoeae* (1), all of which contain glucosamine, almost certainly in the *N*-acetyl form (Tsai and Frasch, unpublished data). The LPS of pathogenic *Neisseria*, like that of *Enterobacteriaceae* species, contains both a lipid A region and a polysaccharide region. The polysaccharide regions of *N. meningitidis* and *N. gonorrhoeae* are devoid of the long O-polysaccharide chains characteristic of smooth LPS (12) and are composed of four sugars: glucose, galactose, glucosamine, and heptose (8). Recent studies in our laboratory indicate that the polysaccharide regions of all meningococcal LPS immunotypes thus far studied contain 15 to 20% glucosamine by weight (C. M. Tsai and C. E. Frasch, unpublished data). However, since WGA also binds *N*-acetyl neuraminic acid, due to structural similarities between this and *N*-acetyl glucosamine, but with a much lower binding constant (11), we treated WGA agglutinable meningococcal strains with neuraminidase and found little effect upon their agglutination in WGA.

Encapsulated meningococcal strains were not agglutinated by WGA. Selection of nonencapsulated variants of these strains rendered them agglutinable by WGA. By analogy, antibody directed against the major outer membrane protein of serotype 2 will not agglutinate an encapsulated serotype 2 organism, but readily agglutinates nonencapsulated type 2 strains (unpublished data).

The failure to identify capsular polysaccharides on *N. gonorrhoeae*, as well as the failure to observe capsules by an electron microscopy India ink technique, (10) strongly suggests that *N. gonorrhoeae* does not have a capsule. The finding that 164 of 165 gonococcal strains tested were strongly agglutinated by WGA (13), when interpreted in light of the present results, provides additional evidence that most *N. gonorrhoeae* strains are nonencapsulated.

Having determined that encapsulated strains of *N. meningitidis* were not agglutinated by WGA, including three group X strains whose capsule consists of a polymer of *N*-acetyl glucosamine-phosphate (3), we examined 34 randomly selected nonserogroupable throat isolates. Unexpectedly, 10 of the isolates failed to agglutinate in the WGA. Two possible reasons for absence of agglutination are: (i) the strains have small amounts of capsular polysaccharide insufficient for strain identification, but sufficient to block WGA agglutination or, (ii) the strains have as yet unidentified capsular polysaccharide(s). Recent experiments in our laboratory indicate that group B meningococcal carrier strains have significantly less capsular polysaccharide than do group B disease isolates (D. E. Craven, M. S. Peppler, C. E. Frasch, L. F. Mocca, P. P. McGrath, and G. Washington, J. Infect. Dis., in press), suggesting that some carrier strains may produce small amounts of capsular polysaccharide. If there are as yet unidentified meningococcal serogroup polysaccharides, the use of WGA to screen nongroupable isolates will enable rapid selection of candidate strains.

Schaeffer et al. have recommended that WGA-mediated agglutination be used for identification of *N. gonorrhoeae* from presumptive gonorrhea patients (13). Our results indicate that WGA is suitable primarily for identification of *N. gonorrhoeae* from the urethra and vaginal or cervical cultures of heterosexual individuals. *N. meningitidis* is not an uncommon venereal disease isolate from homosexual populations (9). Thus, isolates of *N. gonorrhoeae* from pharyngeal sites and those from homosexual men may be confused with nongroupable *N. meningitidis* if one relies on the lectin technique for strain identification.

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