INFECTION AND IMMUNITY, Mar. 1972, p. 346-351
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Vol. 5, No. 3
Printed in U.S.A.

Latex Agglutination Test for Measurement of Antibodies to Meningococcal Polysaccharides

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Received for publication 1 November 1971

A latex agglutination test employing serogroup-specific meningococcal polysaccharides as the antigen has been developed. The test has been used to measure serological responses in patients with meningococcal disease, in meningococcal carriers, and in volunteers who received meningococcal vaccines. It has been shown to be a sensitive and highly specific test for the detection of group-specific meningococcal antibody. The advantages of standardized particles and antigens which are simple to prepare and stable make this assay feasible for many nonspecialized or small laboratories.

Infection with meningococci is followed by the development of group-specific humoral antibodies against the meningococcal organism (1). A variety of serological tests have been developed to measure these antibodies and those induced by polysaccharide vaccines. In a recent paper the specificities, sensitivities, advantages, and disadvantages of many of these tests have been discussed (2).

The present paper describes a new test in which group-specific meningococcal polysaccharide is adsorbed onto latex particles for use in an agglutination test to detect serum antibodies. The use of these inert nonbiological particles as carriers of various antigens offers as its primary advantage a stable, uniform, and easily obtainable vehicle.

MATERIALS AND METHODS

Antigens. Meningococcal strains of group A (A-1), group B (B-11), and group C (C-11) were those previously described (1). The group Y [Boshard (3), WRAIR 6524] strain was isolated from the cerebrospinal fluid of a patient who developed meningococcal meningitis. Purified meningococcal polysaccharides A, B, and C were prepared by the method of Gotschlich et al. (4). Antigens were stored as lyophilized powder in a desiccator jar in the cold or as stock solutions (concentration of 100 or 250 μg/ml) which were held frozen until used.

Crude polysaccharide antigens were prepared by suspending a 6-hr culture (early exponential growth phase) of meningococci grown on chocolate-agar in phosphate-buffered saline (PBS), *p*H 7.2 ± 0.1, for 2 hr (MacFarland $BaSO_4$ standard no. 10). The suspension was then centrifuged at 2,000 rev/min for 15 min, the cells were discarded, and the supernatant fluid was sterilized by filtration through a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.) and stored at −20 C.

Latex particles. A commercial preparation of polystyrene latex particles of uniform particle size and suspension (Latex 0.81, Difco) was used as the antigen carrier.

Serum collection and subjects. Serum specimens were obtained from persons with nasopharyngeal colonization or systemic infection or from vaccinated volunteers and were stored at −20 C until used. Systemic infection was documented by isolation of organisms from blood or cerebrospinal fluid. Meningococci were identified by methods previously described (1). Vaccines used to immunize volunteers have been reported (2).

Sensitization of latex particles. One volume of latex particles in distilled water (3%, v/v) was added to an equal volume of polysaccharide diluted in normal saline or PBS and mixed gently for 30 min at 37 C. The suspension was pelleted by centrifugation at 2,000 rev/min for 15 min, the supernatant fluid was discarded, and the latex particles were diluted 15 times the original volume in PBS, *p*H 7.2 ± 0.1. A grid titration of antigen versus a human serum of high antibody content and one of low antibody content was performed on each new batch of polysaccharide to determine the optimal sensitizing concentration for that antigen.

Latex agglutination (Lx) test. For the Lx test, starting with a 1:2 dilution, serial twofold dilutions of inactivated serum (56 C for 30 min) were made in PBS (*p*H 7.2 ± 0.1) by using disposable microtiter V plates (Linbro Chemical Co., New Haven, Conn.) and 0.025-ml diluters (Cooke Engineering Co., Alexandria, Va.). To each well was added 0.025 ml of sensitized latex particles. The plates were sealed, gently rotated to mix the reagents, and then incubated. The Lx-A, -B, and -C tests were incubated from 4 to 5 hr at 37 C or 16 hr (overnight) at room temperature. The Lx-Y test was incubated for 16 hr at room temperature (overnight). Agglutination patterns were read on a 1+ to 4+ scale, 1+ being considered positive. Positive and negative control sera were included in each test.

Other serological tests. The indirect hemagglutination (HA) (2), fluorescent-antibody (FAB) (2), and radioactive antigen-binding (B. L. Brandt and F. A. Wyle, Bacteriol. Proc., p. 93, 1971) tests were performed as previously described.

Immunoglobulin characterization. To determine which immunoglobulins were responsible for the latex activity, sera were treated with 2-mercaptoethanol (5) or fractionated by sucrose gradient ultracentrifugation (6). The immunoglobulin content of each fraction was determined in a micro-Ouchterlony assay by using monospecific goat antihuman globulins (Hyland, Division of Travenol Laboratories, Inc., Costa Mesa, Calif.).

RESULTS

Standardization of Lx test. To standardize the Lx test, a number of variables was studied for at least one of the antigen.

Effect of pH. The following buffered-saline preparations were studied: glycine, 0.1 M (*p*H 1.4 to 3.6); phosphate, 0.15 M (*p*H 5.1 to 8.2); borate, 0.1 M (*p*H 8.2 to 9.0). The buffers were used throughout the test except for the initial sensitization of latex particles. It was found that the highest agglutination titers for the four latex tests occurred between *p*H 7.0 and 7.2. Therefore, PBS (*p*H 7.2 ± 0.1) was utilized as the standard for each test.

Washing the sensitized particles. Experiments were performed which showed that agglutination titers of group C-sensitized particles by immune sera were not affected by zero, one, or two buffered-saline washes. Thus, the sensitized latex particles were used without the extra washing steps.

Incubation time and temperature. In comparative studies, it was found that the patterns of agglutination with the A, B, and C tests were identical after incubation for 4 to 5 hr at 37 C or 16 to 20 hr (overnight) at room temperature. The Y test, however, gave consistent results only when incubated overnight.

Stability of sensitized latex particles and antigens. Once sensitized, latex particles could be stored at 4 C for the following periods of time: Lx-A, 2 months only; Lx-B and Lx-C, at least 8 months; Lx-Y, at least 2 months. Further studies must be done on the group B, C, and Y Lx tests to determine exactly how long they may be stored. Crude saline antigen preparations have all been stored frozen for at least 9 months without loss of activity.

Effect of heating sera. The effect of heat treatment of antisera was found to be an important variable only with the Lx-A test. When freshly drawn serum from vaccinated volunteers was used, high prevaccination titers were frequently observed. Thus, some antibody rises were masked. Prevaccination serum titers were often significantly reduced by inactivating the sera for 30 min at 56 C. The 2-week postvaccination antibody titers were essentially unaffected. The results of similar experiments with early and late sera from patients with B, C, or Y infections or C vaccination showed no consistent effects of heating although occasional paired sera demonstrated identical titer changes. Freeze-thawing of sera three of more times accomplished the same effect as heating at 56 C. To provide a standardized assay, heat treatment of sera was instituted for each of the group-specific tests.

Antigen standardization. Standardization of the antigens was done by using sera with high and sera with low antibody content since an occasional preparation caused a nonspecific agglutination at higher dilutions (usually greater than 1:128) which obliterated the end point of the high-titered serum.

Properties of antigens used to sensitize latex particles. As different batches of antigens were tested in the latex system, it became apparent that some were much more satisfactory than others. The amount of antigen adsorbed to the latex was considered to be a probable factor. To test this hypothesis, different lots of serogroup A and B antigens were taken at various stages of purification and were tested in a grid titration against sera of known antibody content. The cruder antigens were found to be more active than the purified polysaccharides (Table 1). To assure that the antibody being measured was directed against the polysaccharide, inhibition tests were performed. It was found that the purified polysaccharides, A-5:pure and A-9:pure, completely inhibited the Lx-A tests in which the cruder polysaccharide was used as antigen (A-5:crude, A-9:crude, respectively). Furthermore, purified C polysaccharide made by Gotschlich's method (4) completely inhibited agglutination of latex particles sensitized with a crude saline extract of group C antigen when tested with known immune human serum.

In a second set of experiments, a radioactive group A polysaccharide was prepared by using ^{14}C-sodium acetate. This material was processed into two fractions: a "crude" preparation prepared by precipitating the polysaccharide with 66% (v/v) ethyl alcohol and removing the nucleic acids with one-tenth saturated sodium acetate, and a more purified fraction made by precipitating a portion of this crude preparation with 80% (v/v) ethyl alcohol. Because of the small amount of material prepared, only the protein content could be measured. The crude preparation was found to contain 240 μg of protein/ml, whereas the more purified fraction contained only 25 μg of protein/ml.

A grid titration of a known serum against two-

TABLE 1. *Antibody titers (log 2) of immune sera tested against group A and group B polysaccharides of various degrees of purification*

Determination	A-5 crude	A-5 pure	A-9 crude	A-9 intermediate	A-9 pure	B-36 crude	B-36 intermediate	B-36 pure
Chemical characterization								
Protein	19.3[a]	0.92	37.6	1.8	0.45	47.3	14.5	0.57
Nucleic acid	3.1[a]	0.64	12.0	1.5	0.44	6.6	1.6	0.72
Phosphorous	5.0[a]	7.29	3.76	6.53	—[b]	—	—	—
Sialic acid[c]	—	—	—	—	—	52	91.0	99
Sensitizing dose (mg/100 ml)								
1	5[d]	<1	4	3	<1	7	3	<1
0.5	5	<1	4	2	<1	—	—	—
0.25	5	<1	4	1	<1	—	—	—
0.125	3	<1	4	1	<1	—	—	—

[a] Per cent total dry weight.
[b] Not tested.
[c] Compared to a standard preparation.
[d] Titer of homologous serum (log 2).

TABLE 2. *Grid titration of a known serum against latex particles coated with group A, ^{14}C-labeled polysaccharide taken at different stages of purity*

Antigen[a] dilution	Serum (log 2) titer against indicated antigen	
	Crude prepn	Pure prepn
1:1	4	3
1:2	4	3
1:4	5	2
1:8	4	1
1:16	4	<1
1:32	3	<1
1:64	2	<1
1:128	1	<1

[a] Used to sensitize the latex particle.

fold serial dilutions of labeled antigen was performed (Table 2). It was found that the cruder antigen was active when diluted as much as 1:128 compared to 1:8 for the purified antigen.

Latex particles sensitized at an antigen dilution of 1:1 were also collected on a Millipore 0.45-μm membrane filter, washed, and dried. The activities of the latex particle plus filter paper, filtrate, and a filter-paper control were measured in a Packard Tri-Carb liquid scintillation counter. The per cent of antigen bound to the latex was then determined. It was found that 16 times more crude antigen remained bound to the latex than pure antigen (0.839% versus 0.053%).

Finally, in a series of 57 adult volunteers who received the group C vaccine, the mean antibody increase between the prevaccination and 2-week postvaccination sera using the pure C polysaccharide antigen was 4.08 tubes. Whereas, the same samples tested against latex sensitized with the crude polysaccharide resulted in a 5.86 tube mean difference.

TABLE 3. *Specificity of latex test in patients with meningococcal meningitis*

Patient	Serogroup meningococcus isolated	Day of disease	Serum titer vs. indicated latex antigen			
			A	B	C	Y
GL	Y	1	<1[a]	2	3	<1
		10	<1	2	3	5
SU	B	1	<1	<1	<1	<1
		7	<1	5	<1	<1
		14	<1	5	<1	<1
WO	C	1	<1	<1	<1	<1
		7	<1	<1	7	<1
		36	<1	<1	5	<1

[a] Number of reactive tubes (serial twofold dilutions).

Specificity. The Lx test with meningococcal polysaccharides as antigens is a highly specific test for detecting antipolysaccharide antibodies. Of 32 cases of meningococcal septicemia or meningitis caused by serogroup B (5), C (22), or Y (5) organisms, 30 showed antibody rises only with those latex particles coated with their homologous serogroup antigens. One case in which Y organisms were isolated from cerebrospinal fluid showed no antibody rise to any of the standard antigens. This same patient also showed no response in tests using latex particles sensitized with a crude polysaccharide preparation made from the isolated infecting organism. Another case, in

TABLE 4. *Specificity of latex agglutination test in volunteers receiving meningococcal polysaccharide vaccines*

Subject	Vaccine	Days post-vaccine	Serum titer vs. indicated latex antigen			
			A	B	C	Y
GI	A	0	2[a]	<1	<1	<1
		14	5	<1	<1	<1
IM	A	0	<1	<1	<1	<1
		14	8	<1	<1	<1
SC	C	0	<1	<1	<1	<1
		14	<1	<1	7	<1
HC	C	0	1	<1	<1	<1
		14	1	<1	5	<1

[a] Number of reactive tubes (serial twofold dilutions).

which a group B organism was isolated from the blood, showed antibody increases to both the B and C polysaccharides. In Table 3 are listed results of a battery of Lx tests performed on sera from representative cases of group B, group C, and group Y disease.

The Lx test was also found to be group-specific in volunteers who received group A or C polysaccharide vaccines. Table 4 shows results from four representative subjects who received 50-μg injections of group C vaccine or 50 μg of group A vaccine. When tested against all four serogroup antigens, all of the individuals showed an increase in antibody titer at 2 weeks to the corresponding homologous polysaccharide antigen only.

Sensitivity. The Lx test is quite sensitive as a measure of group-specific meningococcal infection. In a series of 31 proven cases of group C disease, 26 showed antibody rises. In the five remaining cases, a high unchanging titer was found. Since the date of onset of illness was not known for these five patients, it is possible that the first serum tested may have been obtained after the acute stage and thus the antibody increase would have been missed. Serum of one patient with group C disease showed a prozone phenomenon in the Lx-C test 7 days after hospitalization. In a series of 12 group B cases, 11 showed at least a fourfold rise (range 4- to $\geq$256-fold) within 10 days after hospitalization. One group B patient had a high unchanging titer. Four out of five cases of group Y disease developed antibody rises. The remaining case of Y disease had no detectable latex antibody against any of the serogroup polysaccharides.

Persons who had no clinical evidence of disease but who developed positive nasopharyngeal cultures (carriers) were also tested for development of antibodies. Seventeen of 18 persons who had become group C carriers developed group-specific antibodies as did 8 out of 10 persons who had become group Y carriers. However, only 2 of 24 individuals who had become carriers of group B organisms developed an increase in antibody titer.

Comparison of the Lx and FAB tests. The indirect FAB test, which uses the whole organism fixed onto a glass slide as the antigen, is very sensitive for detecting antibodies in subjects receiving the meningococcal polysaccharide vaccines (1, 2). The Lx and FAB tests were compared in 23 volunteers who received meningococcal polysaccharide vaccines (16 group C vaccine; 7 group A vaccine). Both tests detected antibody increases in the same 17 individuals and no antibody rise in the remaining 6 individuals.

Similar correlations were obtained in five cases (two serogroup B, three serogroup C) of meningococcal disease.

Comparison of the Lx and radioactive antigen-binding assays (ABA). Only the Lx-B and Lx-C tests were compared with the ABA test. Seven group B cases and 12 group B carriers were mutually studied. All of the cases and one of the carriers showed rises in both assays. Eleven group C meningitis cases showed antibody rises by both group C assay systems.

Comparison of Lx and HA tests. The Lx and HA tests using meningococcal polysaccharides as antigens showed 100% correlation in adults with disease, carriage, or vaccination (Table 5). When the same batch of polysaccharide antigen was used in both assays, mean titers were higher in the HA test although the mean change in titer was essentially the same (Table 6).

In an experiment using sera from children who had received group C vaccine, seven individuals

TABLE 5. *Comparison of latex agglutination (Lx) and hemagglutination (HA) tests in adults*

Serogroup and category	No. of subjects	No. of subjects with antibody rises[a]			
		Lx pos. HA pos.	Lx neg. HA neg.	Lx pos. HA neg.	Lx neg. HA pos.
C case	18	15	3[b]	0	0
C carrier	22	20	2	0	0
C vaccine	90	87	3	0	0
B case	9	8	1[b]	0	0
A vaccine	50	46	4	0	0

[a] Fourfold rise considered significant.
[b] High unchanging titers.

TABLE 6. *Comparison of mean titers of latex agglutination (Lx)-A and hemagglutination (HA)-A in 50 volunteers receiving group A polysaccharide vaccine*

Days after vaccination	Mean titer[a] vs. indicated antigen	
	Lx-A	HA-A
0	0.18	3.11
14	2.79	6.00
Mean titer change	2.61	2.89

[a] Number of reactive tubes (serial twofold dilutions).

TABLE 7. *Identification of immunoglobulin causing latex agglutination following group C meningococcal disease*

Day of disease	Latex-C titer (log 2)										
	Whole serum	Sucrose density gradient fractions									
		1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	0	0	0	0	0
7	5	1	2	1	1	0	0	0	0	0	0
10	5	1	2	2	0	0	0	0	0	0	0
		← IgM[a] → (fractions 1–3)			← IgG[a] → (fractions 4–8)						
					← IgA[a] → (fractions 4–7)						

[a] Immunoglobulins G, M, and A.

failed to show antibody response by the HA-C test and Lx-C test with a purified C antigen. All seven showed a fourfold or greater Lx-C response when a crude C antigen (saline extract) was used. These positive results were confirmed by the very sensitive radioactive ABA test.

Immunoglobulins active in the Lx test. Sera from a patient with group C meningococcal disease were examined for immunoglobulins active in the latex system. Results of sucrose density centrifugation (Table 7) and 2-mercaptoethanol treatment showed all the activity to reside in the immunoglobulin M moiety. A second patient tested by 2-mercaptoethanol treatment only also showed immunoglobulin M activity exclusively.

Reproducibility. To determine the reproducibility of the test, 30 consecutive tests on selected sera which were assayed over a period of 1 to 4 months were examined. Tests were performed by two different individuals. It was found that the Lx-A test varied one dilution six times (0.20 tubes, average difference); the Lx-B and Lx-C tests varied one dilution three times (0.10 tubes, average difference); the Y test varied four times (0.13 tubes, average difference). Thus, a 2-tube change in dilution was considered significant.

Latex variation. Five different lots of commercial Difco latex preparations were tested to determine whether latex variation might be important. No difference was found.

Multiple latex sensitizations. Other experiments have shown the feasibility of sensitizing latex particles with multiple antigens (B, C, and Y simultaneously). For this test, the three antigens were mixed together at their optimum sensitizing doses and incubated with the latex. Homologous serum antibody titers were identical with mono- or multiple-sensitized particles. The A antigen, however, showed loss of sensitivity when added to the other polysaccharides.

DISCUSSION

The Lx test has as its major advantage its simplicity. The polysaccharide antigen can be extracted very easily from any meningococcal isolate by simply suspending 6-hr-old cultures in PBS, centrifuging, and using the supernatant fluid as the antigen.

Latex particles of known size can be purchased and sensitized without further treatment. The use of such particles avoids the variables found in human or animal erythrocytes which change surface characteristics upon storage, often settle unpredictably, and may require preabsorption of the sera to be tested. Polysaccharide-sensitized latex particles retain their properties when stored for 2 months (in the case of the A polysaccharide) or longer (in the case of B, C, and Y polysaccharides). Thus, large batches of antigen may be prepared and used for relatively long periods of time.

In terms of sensitivity and specificity, the Lx test is quite similar to the HA test for meningococcal antibodies. The group A and B HA tests and the group A and B Lx tests have shown differences in antibody titers of normal and postvaccination human sera even when the same lot of polysaccharide was used for both assays. However, mean titer increases were approximately equal in both assay systems.

With respect to sensitivity, the Lx test is equal to the FAB test when antipolysaccharide antibody is being measured. The FAB test measures antibody against other antigens as well as the polysaccharide of the meningococcus, and, therefore, this test is cross-reactive among the various meningococcal serogroups. For example, studies done on sera of carriers showed group-specific latex antibody rises in the great majority of individuals. However, most of these same individuals showed FAB rises to heterologous as well as homologous

serogroup antigens (2). The failure to demonstrate Lx-B rises in 22 of 24 group B carriers was unexpected. Six of these men were also tested by FAB-B and showed significant titer increases with fluorescein-tagged immunoglobulins G and M and antiglobulins. However, only 1 of 12 tested by the group-specific, highly sensitive, radioactive antigen-binding assay showed a group B polysaccharide titer increase. This same subject had a Lx-B antibody rise. Therefore, the lack of antipolysaccharide response to group B carrier infection appears to be a true finding and not the result of an insensitive latex test.

The Lx-B and -C tests also compared very favorably with the highly sensitive, radioactive, antigen-binding assay. However, the Lx test is much simpler to perform.

A major factor in the latex system appears to be the nature of the antigen preparation used. The data presented above suggest that crude polysaccharide preparations bind to latex particles more firmly or in a greater quantity, or both, than highly purified polysaccharides and thus provide a more-sensitive indicator of antibody than the highly purified materials. Whether the protein or nucleic acid is the important "sticky" factor is not yet known. No studies were done to determine how much protein or nucleic acid, or both, was necessary. That the sticky factor is primarily responsible for the ability of the polysaccharide molecule to coat the latex particle and is not involved in the antibody-antigen agglutination was demonstrated by the ability of a highly purified polysaccharide to inhibit the reaction.

ACKNOWLEDGMENTS

W. D. Zollinger, S. Berman, and P. Altieri prepared many of the polysaccharide antigens; B. L. Brandt performed the hemagglutination and radioactive binding assays. H. Schneider provided valuable guidance. B. E. Hoover provided excellent technical assistance.

LITERATURE CITED

1. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. J. Exp. Med. 129:1307–1326.
2. Artenstein, M. S., B. L. Brandt, E. C. Tramont, W. C. Branch, Jr., H. D. Fleet, and R. L. Cohen. 1971. Serological studies of meningococcal infection and polysaccharide vaccination. J. Infect. Dis.
3. Evans, J. R., M. S. Artenstein, and D. H. Hunter. 1968. Prevalence of meningococcal serogroups and description of three new groups. Amer. J. Epidemiol. 87:643.
4. Gotschlich, E. C., T. Y. Liu, and M. S. Artenstein. 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B and group C meningococcal polysaccharides. J. Exp. Med. 129:1349–1365.
5. Deutsch, H. F., and J. I. Morton. 1956. Dissociation of human serum macroglobulin. Science 125:600–601.
6. Kunkel, H. G. 1960. Macroglobulins and high molecular weight antibodies, p. 294. *In* F. W. Putnam (ed.), The plasma proteins, vol. 1. Academic Press Inc., New York.