HUMAN IMMUNITY TO THE MENINGOCOCCUS

IV. Immunogenicity of Group A and Group C Meningococcal Polysaccharides in Human Volunteers

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The lack of a satisfactory animal model has greatly complicated the study of immunity to systemic meningococcal disease. Mucin-enhanced infection, albeit unsatisfactory, is the only model presently available (1). The studies of Scherp and Rake (2), Watson and Scherp (3), and Kabat et al. (4) with hyperimmune sera of several animal species and convalescent sera of man indicated that antibodies to the group-specific polysaccharides were able to passively protect mice against fatal meningococcal infection. Goldschneider et al. (5) have demonstrated that the presence in human serum of antibodies to meningococcal antigens, as measured by the bactericidal reaction, is strongly correlated with immunity to meningococcal disease. In many of the sera studied, it could be shown that a large part of the bactericidal antibody was directed against the group C–specific polysaccharide (6). These lines of evidence indicate that if antibodies to the group-specific polysaccharides could be induced artificially, they probably would be protective.

Kabat et al. (7) investigated the immunogenicity of the group A polysaccharide in human volunteers. They found it to be a poor antigen. Kabat also injected several human volunteers with the group C polysaccharide prepared by Watson and Scherp (3). The results were equally disappointing (8). These polysaccharides probably all had average molecular weights of less than 50,000 (9).[1]

Work on partially degraded dextrans has indicated that only if the molecular weight of polysaccharides is above 100,000 are the preparations reliably antigenic in human beings (10). Gotschlich et al. (11) have shown that the group-specific A and C meningococcal polysaccharides, when isolated by the method employing the cationic detergent Cetavlon, have high average molecular weights in excess of 100,000. These considerations prompted a reinvestigation of the immunogenicity in human beings of the meningococcal group A and C antigens.

[1] Liu, T. Y., J. K. Jönsen, and E. C. Gotschlich. Manuscript in preparation.

Materials and Methods

Antigens.—The group-specific meningococcal polysaccharides used in this study have been described in the preceding report (11). Meningococcal endotoxins were prepared by the hot phenol-water method of Westphal et al. (12) from group A and group C meningococci. The organisms were grown on the defined agar medium described by Catlin and Schloer (13), harvested in distilled water, centrifuged, and washed with distilled water. They were extracted twice with hot phenol-water mixture. The aqueous layers were pooled; dialyzed extensively against distilled water, and centrifuged at 100,000 *g* for 3 hr. The pellets were suspended smoothly in 30 ml of water and ultracentrifuged for 3 hr at 100,000 *g*. This step was repeated once. The pellet was suspended in a minimal volume of water and lyophilyzed.

Mucin-Enhanced Infection in Mice.—Hog gastric mucin (granular mucin type 1701-W) was purchased from Wilson Laboratories, Chicago, Ill., and prepared as a 5% suspension in water, sterilized by autoclaving, and neutralized with 1 N NaOH before use. Meningococci were grown for 6 hr on Mueller-Hinton agar (14), and a suspension containing approximately 1×10^9 organisms/ml in phosphate-buffered saline was prepared as described by Goldschneider et al. (5). The bacterial suspension was further diluted in mucin and an aliquot was removed and plated to assay the number of viable organisms. Inbred male and female mice of strain C57BL/6 (Microbiological Associates, Walkersville, Md.), weighing 20–25 g were injected intraperitoneally with 1 ml of mucin containing the meningococci and observed for 4 days. Most deaths occurred within 48 hr.

Immunization of Primates.—The immunogenicity of group A polysaccharide lot A-1 and of C polysaccharide lot C-2 was tested in 12 rhesus monkeys weighing about 4 kg each. Three animals were injected with 25 μg of A polysaccharide intradermally, and were reinjected 25 days later with 20 μg of A polysaccharide. At the time of the second injection, they also received 2 μg of C polysaccharide intradermally. Three other monkeys had the same immunization schedule but the order of administration of the antigens was reversed. All animals were bled immediately prior to the first injection and on days 14, 25, and 40 thereafter. Six other monkeys were injected intradermally with 2 μg of the same lots of polysaccharide used above, three with the A polysaccharide and three with the C polysaccharide antigen. These animals were bled 15 and 20 days after immunization. One rhesus monkey weighing about 10 kg was injected intramuscularly with 50 μg of A polysaccharide lot A-1 absorbed onto aluminum hydroxide gel, and was bled 14 and 40 days after injection.

Eight chimpanzees were injected intradermally with 50 μg of polysaccharide, four of them with the A polysaccharide antigen, lot A-5, and the other four with C polysaccharide antigen, lot C-5. Two of the chimpanzees which were injected with A polysaccharide and two injected with C polysaccharide had undergone splenectomy prior to the present study as part of a study of experimental infection with malaria. The other four animals were normal. Bleedings were obtained at the time of injection, and on days 7 and 21 after immunization.

Five gibbons at the SEATO Laboratories in Bangkok, Thailand, were injected intradermally with 25 μg of C polysaccharide, lot C-3, and were bled at weekly intervals for 4 wk.

Toxicity Studies.—The general safety test employing guinea pigs as described in the Public Health Service Regulations (15), Section 73.72, was performed with 500 μg doses of meningococcal polysaccharide, lots C-4, C-5, and A-5. The pyrogenicity of lots C-4, C-5, and A-5 was tested in albino rabbits (about 2.2 kg) as outlined in the Public Health Service Regulations (15), Section 73.74. Interference with the growth rate of weanling mice was assayed by the method of Pittman and Cox (16). The method of Dubos and Schaedler (17) was used to assay for the presence of contaminating endotoxin.

Sterility.—Sterility of the meningococcal polysaccharide prepared for human use (lots C-4, C-5, and A-5) was tested in bulk before final packaging and on 10% of the final packaged material, in accordance with the regulations of the Public Health Service (15), Section 73.73.

Serological Methods.—Passive hemagglutination was performed as described by Gotschlich et al. (11). Polysaccharides from lot A-2 or C-2 were used to sensitize the red cells. The assay for bactericidal antibody and the immunofluorescent assay for antibody were performed as described by Goldschneider et al. (5). Fluorescein-conjugated rabbit sera against the heavy chains of human immunoglobulin A, G, and M were prepared by Behringwerke, Marburg-Lahn, Germany, and purchased from Hoechst Pharmaceutical Co., Kansas City, Mo. These companies also supplied the plates (Partigen) and method used for quantitative radial immunodiffusion for the determination of the amount of the various immunoglobulins in chromatographically separated serum.

Gel Filtration of Human Serum.—1 ml of serum was fractionated by gel filtration on Sephadex G-200 in a column designated K 15/90 (Pharmacia, Inc., Piscataway, N. J.). The protein concentrations in the eluates was determined by the absorbancy at 280 mμ. The buffer used was tris(hydroxymethyl) aminomethane (Tris)-HCl, 0.1 M, pH 8.0.

Human Sera.—Blood obtained from volunteers was allowed to clot at 37°C for 1 hr, centrifuged, and the major part of the serum stored frozen at −73°C. Small aliquots of serum were stored at 4°C. The latter samples were used for passive hemagglutination and for ring tests, whereas the frozen samples were used for all other studies.

Cultures.—Nasopharyngeal cultures to determine the presence of meningococci were performed as described by Artenstein et al. (18). The serological grouping was done by the method of Evans et al. (19).

RESULTS

The Immunogenicity of High Molecular Weight A and C Meningococcal Polysaccharides in Laboratory Animals.—

1. Mice: The antigenicity of the meningococcal polysaccharides was first tested in mice. This species was chosen because there is extensive experience on its response to other polysaccharides, particularly the pneumococcal type-specific antigens (20). The latter work has delineated the optimum dose of polysaccharide to be used for immunization (0.1–1.0 μg) and the moment of maximum immunity (1 wk after immunization) against a challenge infection with living pneumococci (20).

It has been shown that the majority of strain C57BL/6 mice succumb when injected with 10 or more meningococci suspended in 5% hog gastric mucin, and that these mice can be passively protected against fatal meningococcal infection by prior injection of antiserum. Several experiments were performed using this model. The results of one of these studies are shown in Table I.

Mice were injected intraperitoneally with the appropriate polysaccharide. Doses of 0.05, 0.10, and 0.50 μg were employed. 1 wk later, the animals were challenged by intraperitoneal injection with approximately 100 meningococci suspended in mucin. This unusually small dose was chosen to maximize the opportunity of observing active immunity. No protective effect was observed.

In another experiment, mice immunized 1 wk before with 0.5 and 1.5 μg of A polysaccharide, lot A-1, were challenged with 1000 group A meningococci of strain A1. All the animals died. The same result occurred when mice injected with 0.1 and 1.0 μg of C polysaccharide, lot C-1, were challenged with 1000 group C meningococci of strain C11.

2. Primates: 13 rhesus monkeys were injected with various doses of the A and the C polysaccharide as described under Materials and Methods. Sera from the animals were tested for the presence of antibodies with the passive hemagglutination test. No increases in titers were observed.

Four chimpanzees were immunized with 50 μg of A polysaccharide, lot A-5, and four with an equal amount of C polysaccharide, lot C-5. None of the sera obtained from the chimpanzees showed any increases in hemagglutination titers.

Five gibbons were injected intradermally with 25 μg of C polysaccharide, lot C-3. No change in hemagglutination titers were observed in four of these animals. However, one gibbon had a definite immune reaction; its hemagglutination titer rose from 1/8 to 1/128 within 1 wk of immunization and declined to 1/64 over the next 2 wk.

TABLE I

Survivors among Mice Injected with Meningococcal A or C Polysaccharide and Challenged 1 Wk Later with Meningococci Suspended in Mucin

Immunization	Challenge	No. of mice	Survivors
Lot A-5, 0.05 μg	100 A1 meningococci	20	2
Lot A-5, 0.10 μg	" " "	20	4
Lot A-5, 0.50 μg	" " "	20	3
Controls	" " "	20	4
Lot C-5, 0.05 μg	100 C11 meningococci	20	3
Lot C-5, 0.10 μg	" " "	20	2
Lot C-5, 0.50 μg	" " "	20	4
Controls	" " "	20	3

Toxicity of the Group-Specific Meningococcal Antigens in Laboratory Animals. —Four tests to assay the toxicity of meningococcal polysaccharides were carried out in laboratory animals; two were designed to detect contamination with biologically active endotoxin, and the other two were general toxicity tests. Toxicity controls of polysaccharides, lots C-4, C-5, and A-5, prepared expressly for human use, were performed on final packaged material, which had passed the sterility tests.

1. Pyrogenicity in rabbits: A dose of 2.5 μg of C-4, C-5, or A-5 was injected intravenously into each of three albino rabbits. As a positive control, other rabbits were injected with 0.010 μg of endotoxin isolated from the same meningococcal strains (A1 and C11) used for the preparation of the group-specific polysaccharides. Rectal temperatures were obtained on these animals. The results are set forth in Table II.

The nine animals injected with the polysaccharide preparations did not

exhibit any perceptible pyrogenic responses. However, the six animals injected with 0.010 μg of endotoxin from strain A1 or C11 had definite febrile responses. This experiment indicates that the polysaccharides contained less than 1% by weight of biologically active endotoxin.

2. Inhibition of drinking by mice: The absence of significant amounts of endotoxin was also demonstrated by the test described by Dubos and Schaedler (17). Normal mice gain weight overnight primarily because of water intake. However, animals injected with endotoxin do not drink normally and show a

TABLE II

Temperature Response of Rabbits to the Intravenous Injection of Meningococcal Polysaccharides or Endotoxins

Rabbit	Inoculation	Initial	½ hr	1 hr	1½ hr	2 hr	3 hr
1	Polysaccharide, lot C-4,	39.5	39.4	39.3	39.4	39.4	39.3
2	2.5 μg	39.0	39.0	39.0	39.0	39.0	38.8
3		39.0	38.8	38.7	38.6	38.7	38.7
4	Polysaccharide, lot C-5,	39.0	38.7	38.9	38.8	38.7	38.6
5	2.5 μg	39.9	39.8	39.8	39.8	39.6	39.5
6		39.0	39.0	38.8	38.7	38.6	38.5
7	Endotoxin, strain C11,	39.8	40.0	40.3	40.4	40.3	40.1
8	0.01 μg	39.6	39.9	40.7	40.8	40.5	40.3
9		39.0	39.1	40.2	40.8	40.3	40.1
10	Polysaccharide, lot A-5,	39.1	39.0	39.1	39.1	39.4	39.3
11	2.5 μg	39.2	39.2	39.3	39.3	39.3	39.2
12		39.0	39.1	39.3	39.3	39.3	39.3
13	Endotoxin, strain A1,	38.7	38.4	39.6	39.9	40.1	39.6
14	0.01 μg	38.7	38.7	39.5	39.9	39.8	39.4
15		38.1	38.0	39.9	39.9	39.7	39.4

weight loss overnight. Mice were weighed and injected at 4 p.m. with 100 μg of polysaccharide or with either 1.0 or 0.5 μg of meningococcal endotoxin. Water and food were supplied ad lib. The animals were weighed the next morning and the average weight change per mouse was calculated. The results of four experiments are shown in Table III.

The mice injected with polysaccharide generally gained less weight than control animals injected with saline. The injection of one preparation, lot C-3, caused a loss of weight. However, in no experiment did the mice injected with polysaccharide lose as much weight as the animals injected with 0.5 μg or 1 μg of endotoxin

3. Growth of weanling mice: One of the general toxicity tests employed determined the effect upon the growth rate of weanling mice resulting from the injection with meningococcal polysaccharides (16). Male or female albino mice weighing 14–16 g were injected with 100 μg of the various lots of polysaccharide, and their weights were observed for 1 wk. Table IV indicates the average weight gain per mouse over the 7 day period. It should be noted that the female mice

TABLE III

Average Overnight Weight Change of Mice Inoculated with Meningococcal Polysaccharides or Endotoxins

Exp.	Test substance	No. of mice injected	Weight gain per mouse
			g
1	Saline control	15	+1.40
	Lot A-1, 100 μg	5	+1.60
	Lot A-2, 100 μg	5	+1.00
	Lot C-2, 100 μg	5	+0.30
	A endotoxin, 1.0 μg	5	−0.52
	C endotoxin, 1.0 μg	5	−1.20
2	Saline control	15	+0.93
	Lot C-3, 100 μg	15	−0.87
	Lot A-3, 100 μg	15	+0.87
	C endotoxin, 0.5 μg	15	−2.24
3	Saline control	20	+1.20
	Lot C-4, 100 μg	15	+0.92
	Lot C-5, 100 μg	15	+0.88
	C endotoxin, 0.5 μg	15	−1.98
4	Saline control	20	+0.42
	Lot A-5, 100 μg	20	+0.31
	A endotoxin, 0.5 μg	20	−0.58

used in the third and fourth experiments gained weight more slowly than males. The experiment was done twice with lot A-5. The growth rate of the mice injected with polysaccharide was no different than that of animals injected with saline.

4. Toxicity for guinea pigs: The other general safety test is one required by federal regulations for biological products (15). Three guinea pigs weighing about 350 g were injected with 500 μg of each lot of polysaccharide and their weights and temperatures were observed for 1 wk. The lots of polysaccharide C-4, C-5, and A-5 were tested by this method. The animals showed normal weight gain and no febrile responses.

The Immunogenicity of High Molecular Weight A and C Meningococcal Polysaccharides in Human Volunteers.—The immunogenicity of the group-specific polysaccharides was first tested in one volunteer and then in an additional five. All of the volunteers were males ranging in age from 24 to 38 yr, and were laboratory personnel. One of the subjects was a Negro, the others Caucasian. Throat cultures were obtained at the beginning of the study, at weekly intervals for 4 wk and occasionally thereafter, and were examined for the presence of meningococci (18, 19). All cultures were negative except for subject I.G. who had

TABLE IV

*The Effect of Meningococcal Polysaccharides on the Growth Rate of Weanling Mice**

Exp.	Test substance	No. of mice	Weight gain per mouse
			g
1	Saline control	20	+8.32
	Lot C-4, 100 μg	20	+8.30
	Lot C-5, 100 μg	20	+8.12
2	Saline control	25	+9.00
	Lot A-5, 100 μg	20	+7.60
3	Saline control	40	+4.52
	Lot A-5, 100 μg	25	+5.07
4	Saline control	10	+6.10
	Lot A-2, 100 μg	10	+6.00
	Lot A-3, 100 μg	10	+6.50
	Lot C-2, 100 μg	10	+6.10
	Lot C-3, 100 μg	10	+5.90

* Experiments 1 and 2 were done with male mice; Experiments 3 and 4 with female mice.

recently acquired a group B meningococcus. Subject J.W. was known to have carried group C meningococci until about 4 months prior to this study.

The subjects were bled and then injected intradermally in the forearm with 0.20 ml of isotonic saline containing 50 μg of polysaccharide. The dates and the sequence of immunizations are indicated in Table VII. The subjects were asked to report any signs or symptoms of toxicity and to measure their temperature 8 hr after the injection. None of the volunteers had any signs of systemic toxicity and no febrile responses occurred.

All subjects showed a skin reaction to the injections. There were no immediate wheal and erythema reactions. About 4 hr after the injection, a pale orange discoloration occurred which was limited to the site of the bleb. One subject (E. C. G. injected with A-2) reported mild itching locally which disappeared

within 6 hr. At about 8 hr, all subjects reported an area of erythema and sometimes slight induration around the site of injection. The reactions ranged up to 50 mm in diameter, were slightly tender, and reached maximum dimensions by 20 hr. They then faded completely over the next 48 hr, leaving in most instances an area of pigmentation about 10 mm in diameter which disappeared in about 2 wk.

Serum specimens were collected at weekly intervals for 4 wk and occasionally thereafter. Antibody responses were measured by several methods.

1. Hemagglutinating antibodies: Results in Tables V and VI show that all subjects responded with the production of hemagglutinating antibody within 1 wk of injection of polysaccharide and reached peak titers within 2 wk. The

TABLE V

*Reciprocal Hemagglutination Titers of Six Subjects Immunized with Meningococcal C Polysaccharide**

Subjects	Weeks following immunization with C antigen							
	0	1	2	3	4	6	20	35
M. S. A.	2	64	256	256	256	256	512	
W. C. B.	2	32	256	256	256		256	
I. G.	2	32	128	128	128	128	128	
E. C. G.	2	128	256	128			128	128
J. S.	2	64	64	64	64	64	64	
J. W.	32	64	128	128	128	128	128	

* Cells sensitized with C polysaccharide.

geometric mean of the reciprocal titers was 161 for the response to the C polysaccharide, and 446 for the response to the A polysaccharide. The titers remained unchanged or dropped one 2-fold dilution during the remainder of the study. Subject J. W. was known to have been a group C carrier until a few months before this study, and this probably accounts for his initial titer of 1/32 against red cells sensitized with the C antigen. Due to the nature of the hemagglutination system, the other initial titers should not be interpreted as indicating either the presence or absence of antibodies. What is of interest are the pronounced increases in titer following the injection of the antigens.

2. Precipitating antibodies: The sera of these subjects were also tested qualitatively for the presence of precipitating antibody employing the ring test. All subjects except J. S. had detectable precipitating antibodies to the A and the C polysaccharide within 2 wk after injection with the respective antigen. The sera of subject J. S. did not at any time produce a positive ring test with either antigen.

3. Bactericidal antibodies: The bactericidal antibody response resulting from

the immunizations was tested by measuring the bactericidal activity of the sera against four test strains of meningococci in the presence of an exogenous source of human complement. Two of the test organisms belonged to group A and the other 2 to group C. The results are set forth in Table VII.

All subjects, except E. C. G., were injected first with the C polysaccharide. Their bactericidal activities to the group C test organisms rose to full titer within a week or two. Subject J. W., who had been a group C carrier and had a very high initial bactericidal titer to the group C organisms, nevertheless experienced a considerable rise in activity after the injection with C polysaccharide. None of the subjects had increases in bactericidal activity to the group A test organisms as a result of injection with the C polysaccharide, but all volunteers did produce bactericidal antibody to the group A test organisms in

TABLE VI

*Reciprocal Hemagglutination Titers of Five Subjects Immunized with Meningococcal A Polysaccharide**

Subjects	Weeks following immunization with A antigen					
	0	1	2	4	18	37
M. S. A.	8	128	1024	1024	512	
I. G.	32	128	512	512	256	
E. C. G.	8	128	512	512	256	256
J. S.	16	64	128	128	64	
J. W.	16	512	512	512	512	

* Cells sensitized with A polysaccharide.

response to injection with the A polysaccharide. Subject W. C. B. was not injected with the A polysaccharide and never developed increased bactericidal activity to the group A test organisms. Subject I. G. showed the same pattern of increases in bactericidal response even though he was a carrier of group B meningococci.

The order of the immunizations was reversed for subject E. C. G. and it should be noted that injection with the A polysaccharide did not increase the bactericidal activity against the group C test organisms. The bactericidal activity of the sera obtained from this subject was tested against a greater number of meningococcal strains and the results are presented in Table VIII.

After immunization with the polysaccharide antigens, there were demonstrable increases in bactericidal activity to all strains of meningococci belonging to group A or group C. However, no increases in bactericidal activity occurred to organisms belonging to group B or to 135 (19).

Nature of the Antibody Response to Meningococcal Group Specific Polysaccharides.—The classes of antibody produced in the blood of human volunteers after in-

TABLE VII

Serum Bactericidal Antibody in Six Subjects after Immunization with Meningococcal Group-Specific Polysaccharides

Subject	Immunogen	Reciprocal bactericidal titer against			
		Group A strains		Group C strains	
		A1	121 misc.	C11	107 VI
M. S. A.					
5 April	C-5, 5 April	4	4	16	16
12 "		4	4	128	512
19 "	A-5, 19 April	4	4	2048	1024
26 "		32	32	2048	1024
3 May		128	256	2048	1024
13 August		256	256	2048	2048
W. C. B.					
1 April	C-5, 5 April	4	4	8	8
12 "		4	4	16	64
18 "		4	4	128	256
26 "		4	4	128	256
3 May		4	8	128	256
13 August		4	4	128	256
I. G.					
2 April	C-5, 5 April	32	32	16	16
12 "		32	16	32	64
19 "	A-5, 19 April	32	16	256	512
26 "		32	128	256	256
3 May		64	256	256	512
17 May		64	256	256	512
13 August		128	256	256	512
E. C. G.					
30 November	A-2, 30 November	4	8	8	4
7 December		8	16	8	
11 "		64	128		
14 "	C-2, 14 December	64	128	8	4
21 "		64	128	256	256
28 "		64	128	256	512
5 January		64	256	256	512
13 August		128	256	128	512
J. S.					
2 April	C-5, 5 April	8	8	4	4
12 "		8	8	64	64
19 "	A-5, 19 April	8	16	256	256
26 "		32	64	256	256
3 May		32	128	256	256
17 "		32	64	512	256
26 August		32	32	256	256

TABLE VII (*Concluded*)

Subject	Immunogen	Reciprocal bactericidal titer against			
		Group A strains		Group C Strains	
		A1	121 misc.	C11	107 VI
J. W.					
2 April	C-5, 5 April	8	8	128	256
12 "		8	8	256	1024
19 "	A-5, 19 April	8	8	1024	1024
26 "		128	128	1024	1024
3 May		256	256	1024	1024
17 "		256	256	1024	1024
13 August		256	256	512	512

TABLE VIII

Bactericidal Activity of Preimmune and Immune Serum of Subject E.C.G. against Various Meningococcal Strains

Meningococcal strain	Serogroup	Reciprocal bactericidal titer	
		ECG, November 30	ECG, February 21
A-1	A	4	64
120-Misc	A	8	64
121-Misc	A	8	128
C-11	C	8	256
182-I	C	8	256
91-II	C	8	128
176-III	C	8	256
158-IV	C	8	128
140-V	C	8	128
107-VI	C	4	512
85-III	B	8	8
122-Misc	135	8	8
166-IV	B	8	8
169-III	B	4	4
153-I	B	8	8

jection with the meningococcal group-specific polysaccharides were studied by indirect immunofluorescence. IgG, IgM, and IgA antibodies were specifically identified using fluorescein-conjugated rabbit antisera prepared against the heavy chains of human immunoglobulins. Antibody titrations were done, using group A or group C meningococci, according to the method of Goldschneider et al. (5).

TABLE IX

Semiquantitative Determination of the Amount of Antimeningococcal Antibodies in Each Immunoglobulin Class in the Sera of Six Volunteers Immunized with Meningococcal Group-specific Polysaccharides.

Subject	Immunogen	Reciprocal immunofluorescence titers with antibodies to human immunoglobulins					
		Immunoglobulin G		Immunoglobulin M		Immunoglobulin A	
		A1*	C11*	A1	C11	A1	C11
M. S. A.							
5 April	Lot C-5, 5 April	<4	4	<4	<4	2	<4
12 "		<4	64	<4	16	2	32
19 "	Lot A-5, 19 April	<4	256	<4	32	2	256
26 "		32	256	16	64	32	256
3 May		256	256	128	64	128	256
13 August		128	256	64	64	64	128
W. C. B.							
1 April	Lot C-5, 5 April	4	4	8	4	8	8
12 "		4	32	8	32	8	8
18 "		4	128	8	128	8	128
26 "		4	256	8	128	8	128
3 May		4	128	8	128	8	128
13 August		4	128	4	64	8	64
I. G.							
2 April	Lot C-5, 5 April	16	16	8	8	16	8
12 "		32	64	16	32	32	32
19 "	Lot A-5, 19 April	32	128	16	64	32	128
26 "		256	128	64	64	128	128
17 May		256	128	64	64	128	128
13 August		256	128	64	32	64	64
E. C. G.							
30 November	Lot A-2, 30 November	4	4	<2	2	<2	<2
11 December		32	—	8	—	16	—
14 "	Lot C-2, 14 December	64	4	16	2	32	<2
21 "		64	—	32	—	32	—
28 "		128	64	16	32	16	32
18 January		128	64	32	32	32	16
21 February		64	128	32	32	32	32
13 August		128	64	32	32	32	32

* A1 and C11 refer to a group A and group C meningococcal strain respectively. These organisms were allowed to dry on microscope slides, exposed to 2-fold dilutions of the human sera, and stained with antisera to human immunoglobulin G, M, and A.

TABLE IX (*Concluded*)

Subject	Immunogen	Immunoglobulin G		Immunoglobulin M		Immunoglobulin A	
		A1	C11	A1	C11	A1	C11
J. S.							
2 April	Lot C-5, 5 April	8	4	<4	4	<4	4
12 "		8	64	<4	32	<4	16
19 "	Lot A-5, 19 April	16	64	<4	32	<4	32
26 "		32	128	32	128	64	128
3 May		64	128	32	128	128	128
17 "		64	128	32	128	64	128
26 August		64	128	32	64	32	64
J. W.							
2 April	Lot C-5, 5 April	16	32	8	16	8	4
12 "		16	32	8	64	8	16
19 "	Lot A-5, 19 April	16	128	8	64	8	128
26 "		128	256	32	64	64	128
3 May		256	256	256	64	256	128
17 "		256	256	256	64	256	128
13 August		256	256	128	32	128	64

As a result of the injection with the C polysaccharide, the sera of subjects M. S. A., W. C. B., J. S., and J. W. contained significantly increased amounts of antibodies binding to group C meningococci, but not to group A organisms (see Table IX). Antibodies belonging to each of the three major classes of immunoglobulin were produced. After injection with the A polysaccharide, similar antibodies capable of binding to group A meningococci appeared. The same was true for subject E. C. G., except that the order of the immunizations was reversed.

It should be remembered that this method, while specific in identifying what class of antibody is bound to the meningococcal cells, does not identify the antigen to which the antibody is bound. This may be pertinent in the interpretation of the antibody response of subject I. G. who was a carrier of group B meningococci during the study. His response does not appear to differ significantly from the responses of the other subjects who were immunized by the same schedule. It is, however, possible that the slight rise in titer of antibody to group A organisms which occurred prior to injection with A polysaccharide was due to cross-reactive antibodies resulting from his concurrent nasopharyngeal infection.

The Immunoglobulin Class of the Antibodies Responsible for the Bactericidal Activity.—Goldschneider et al. (6) have shown that in serum from two normal adults the majority of the antibodies bactericidal to meningococci belonged to

the immunoglobulin G class. They also demonstrated that the bactericidal spectrum of maternal and cord serum was identical, further evidence that immunoglobulin G antibody is primarily responsible for the meningococcocidal activity, since cord serum contains essentially only this kind of antibody (5).

In order to determine whether the artificially induced bactericidal activity mimicked the natural antibody response in this regard, an immune serum obtained from subject E. C. G. 37 wk after immunization with the A antigen was fractionated by gel filtration on Sephadex G-200. The elution profile is shown in Fig. 1. The contents of the odd tubes were sterilized by filtration through

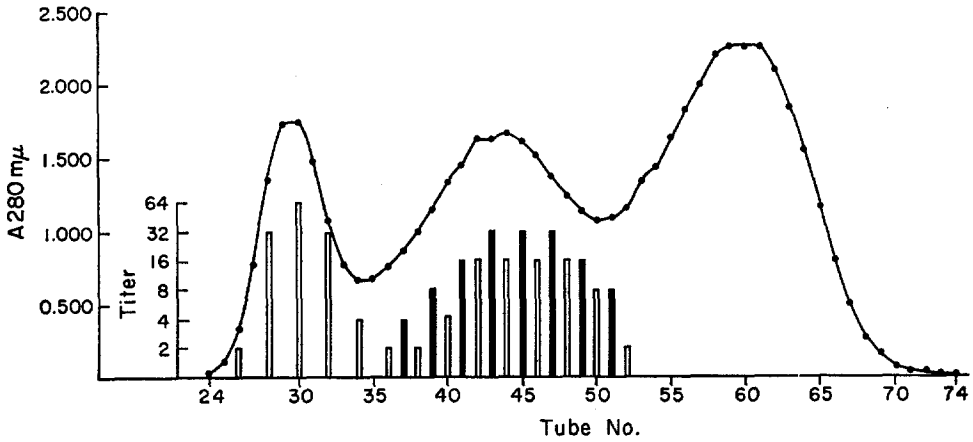

FIG. 1. The separation of human immune serum by Sephadex G-200 gel filtration. The elution profile represents the absorbance at 280 mμ. The open bars indicate the hemagglutinating activity; the solid bars indicate the bactericidal activity in the presence of exogenous complement.

Millipore filters and assayed for their ability to kill group A meningococci in the presence of exogenous complement. The eluates in the even tubes were assayed for the presence of hemagglutinating antibody. The bactericidal and hemagglutination titers are also entered in Fig. 1.

The hemagglutinating activity was separated into two peaks, whereas the bactericidal activity was found solely in the second peak. The eluates in tubes 30, 48, and 60 were assayed for their content of immunoglobulin A, G, and M by quantitative radial immunodiffusion and these results are indicated in Table X.

It is clear from the elution profile and further confirmed by the results set forth in Table X that the bactericidal activity of this serum could be recovered in the immunoglobulin G fraction. The first peak containing immunoglobulin A and M had good hemagglutinating activity but no bactericidal power.

It should be mentioned that this serum also contained antibodies to the group C polysaccharide, and that the eluates were tested for bactericidal activity against a group C test organism, and for hemagglutinating activity with red cells coated with C polysaccharide. Surprisingly, both of these activities were completely absent. It was then realized that the fractionating column had been used previously to assay the gel filtration behavior of a preparation of group C polysaccharide, and evidently enough antigen had remained adsorbed to the column to completely remove all antibodies directed to this polysaccharide.

TABLE X

*The Concentration of IgA, IgG, and IgM in the Eluate of Chromatographically Separated Human Immune Serum**

Tube No.	IgG	IgM	IgA
	mg/100 ml‡	*mg/100 ml*	*mg/100 ml*
30	<5	21	9
48	85	<3	<3
60	<5	<3	<3

* See Fig. 1.

‡ The concentrations of immunoglobulins were measured by radial immunodiffusion.

DISCUSSION

More than a score of large scale attempts have been made to develop a vaccine to combat meningococcal meningitis (21). Several of the vaccines were clearly ineffective, while others yielded inconclusive results. A vaccine prepared at the Pasteur Institute in Brazzaville from 1936 to 1939 was apparently successful. Unfortunately, the material produced by the same institute after World War II was no longer effective (21). More recently, Greenberg and Cooper (22) produced a soluble vaccine which has protective power for mice.

The present study, which tests the antigenicity of high molecular weight group-specific meningococcal polysaccharides in man, represents a first step in the development of these polysaccharides as possible prophylactic immunizing agents. In the course of this study, the immunogenicity of four preparations of meningococcal polysaccharide were tested. None of these materials possessed significant toxicity for laboratory animals and no untoward reactions occurred among the six human volunteers. Subject J. W. had been a carrier of group C meningococci until 4 months preceding the study and had circulating antibodies to group C polysaccharide. His reaction to injection with group C polysaccharide did not differ from the other volunteers. Both the A and the C polysaccharides proved to be good immunogens in man. This result is at variance with previous experiments (7, 8), but the polysaccharides tested prior to the present study were probably less than 50,000 average molecular weight (9).[1] It is our

belief that the high molecular weight of the polysaccharides used in this study accounts for their immunogenicity, a hypothesis, which, however, has not been tested directly.

Antibodies belonging to each of the three major classes of immunoglobulins were produced in response to a single intradermal injection with 50 μg of A or C polysaccharides. The sera of the six human volunteers became highly bactericidal after immunization, killing all strains of group A or group C organisms tested. Peak titers were reached within 2 wk and the levels of bactericidal activity remained essentially unchanged for 5 months. This finding is in agreement with the experience that the human precipitating antibody response elicited by purified polysaccharides is long lasting. Heidelberger (23) reports that detectable antibody to type-specific pneumococcal polysaccharide was usually still present 3 yr after immunization and frequently as late as 8 yr.

It was not possible to induce active immunity in mice with meningococcal polysaccharides, whereas this is readily accomplished with pneumococcal type-specific polysaccharides (20, 24). It is not known whether this finding indicates that the meningococcal polysaccharides are not antigenic in the mouse, or that the deficiencies of the mucin-enhanced infection as an assay system negate the opportunity to observe an immune response. More sensitive methods for the detection of antibodies will have to be employed to settle this issue. An interesting development in this area is the recent study of Siskind et al. (25) employing isotopically labeled pneumococcal polysaccharide to measure quantitatively the immune response of the mouse. They found the greatest concentration of antibody about 1 wk after immunization and a slow decline thereafter. Their data indicate that the average immune response of the mouse is about 10 times less than the average human response to this antigen (26).

The present study has also tested the immunogenicity of the meningococcal polysaccharides in several primate species. There was no evidence for antibody production in rhesus monkeys or in chimpanzees. One out of five gibbons responded, but the interpretation of such an isolated response is difficult. Study of the immunogenicity of meningococcal polysaccharides in this species should be pursued further.

A new immunizing agent which is to be considered seriously for field trials should have certain attributes, among which are immunogenicity and lack of significant toxicity. Furthermore, there need be indications that the antibodies elicited are likely to be protective. It is felt that the present report shows that the high molecular weight group-specific meningococcal polysaccharides fulfill these criteria and hence merit further attention to assess their value as prophylactic vaccines.

SUMMARY

High molecular weight group A and group C meningococcal polysaccharides had no significant toxicity for mice or guinea pigs. Furthermore, these polysac-

charide preparations contained negligible amounts of biologically active endotoxin.

The high molecular weight group A and group C meningococcal polysaccharides were excellent immunogens in six human volunteers. Antibodies belonging to the immunoglobulin classes IgG, IgM, and IgA were produced.

These antibodies were highly meningococcocidal in the presence of complement.

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