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Clinical and Laboratory Investigations of Monovalent and Combined Meningococcal Polysaccharide Vaccines, Groups A and C (39563)

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Highly purified group A and C meningococcal polysaccharide vaccines were developed at the Walter Reed Army Institute of Research, principally by Gotschlich, Artenstein, and Goldschneider (1-3). These vaccines have proved highly safe and effective (1-10) in studies in man, and vaccines of commercial source have been licensed for use in the United States and elsewhere.

The present report summarizes the clinical and laboratory findings in studies in adult subjects given one or more doses of single or combined meningococcal vaccines prepared in these laboratories. Additionally, data relating to stability of the vaccine on prolonged storage in dried or liquid form at various temperatures and to the use of thimerosal preservative in the diluent are presented.

Materials and methods. Vaccines. The vaccines were formulated in the Merck Sharp & Dohme Research Laboratories, West Point, Pa., employing purified polysaccharide antigens prepared by Dr. Thomas H. Stoudt and his associates of the same institute, but at the Rahway, N. J., site. The polysaccharides were assayed for their physical and biochemical attributes, and the final products were considered satisfactory for use in human beings after they passed the usual tests for release of product. All lots were in accord with the current standards under which these products are released for commercial distribution by the Bureau of Biologics, U.S. Food and Drug Administration. Each antigen was present in a 50-μg amount per 0.5-ml dose given sc.

Clinical studies. The subjects were persons in open populations in suburban Philadelphia or in the environs of San Jose, Costa Rica. Informed written consent was obtained. Blood samples were drawn immediately prior to and 2 or 4 weeks after a single dose of vaccine. Modified regimens were used when multiple vaccine doses were given. The patients were observed for local and systemic reactions 4 hr, and 1, 2, 3, and 10 days after vaccinations. Observations were made by physicians or nurses.

Bactericidal antibody assays. The sera were stored frozen at $-20°$ until tests for homologous serum bactericidal antibody were performed using a modification of a procedure (11) employing adsorbed adult rabbit complement. A fourfold or greater increase in serum antibody titer following vaccination was considered to be significant. The lowest dilution of serum tested was 1:2.

Results. Monovalent and combined vaccines given in a single dose. Groups of 25 or 26 persons in the United States (Study No. 404) or 15 persons in Costa Rica (Study No. 400) were given a single dose of group A, C, or combined A-C meningococcal polysaccharide vaccine. Serum bactericidal antibody responses are given in Table I. The responses are expressed both as proportion of persons showing fourfold or greater increase in antibody titer and as geometric mean titer. The large majority of persons was without detectable antibody before vaccination. It is seen that almost all persons in the United States responded to vaccination with a fourfold or greater antibody increase, while 80% or more of the persons in Costa Rica showed such response. The significant finding was that there was no important dif-

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TABLE I. SERUM BACTERICIDAL ANTIBODY RESPONSES IN HUMAN SUBJECTS MEASURED 4 WEEKS FOLLOWING A SINGLE DOSE OF MENINGOCOCCUS A, C, OR COMBINED A-C VACCINE.

Study no.	Vaccine given	Antibody response against Meningococcus A: Initial seropositive ≥4× Rise No./total[a]	%	G.M.[b]	Initial seronegative ≥4× Rise No./total	%	G.M.	Total group ≥4× Rise No./total	%	G.M.	Meningococcus C: Initial seropositive ≥4× Rise No./total	%	G.M.	Initial seronegative ≥4× Rise No./total	%	G.M.	Total group ≥4× Rise No./total	%	G.M.
404 USA 19–62 years	Mono A (D365)	4/4	100	304	22/22	100	66	26/26	100	84									
	Mono C (D367)										0/0	-		24/25	96	51	24/25	96	51
19–62 years	Combo AC (D369)	5/5	100	97	20/20	100	64	25/25	100	71	4/4	100	152	22/22	100	80	26/26	100	88
400	Mono A (D365)	8/10	80	91	4/5	80	16	12/15	80	51									
Costa Rica	Mono C (D367)										3/4	75	38	10/11	91	39	13/15	87	38
19–69 years	Combo AC (D369)	2/3	67	161	11/12	92	45	13/15	87	58	3/3	100	64	11/12	92	29	14/15	93	34

[a] Number showing rise/total number tested.
[b] Geometric mean titer.

ference in response to the components of the vaccine when given in combination compared with the same vaccine given singly, either in height of antibody or percentage with fourfold or greater increase in antibody titer.

The antibody responses found in groups of persons given three different lots of combined meningococcus A and C vaccines in a further study are shown in Table II. The antibody responses to the combined vaccines were roughly the same as found in Study No. 404 above (see Table I). This showed the consistency of response obtained when different lots of vaccine were used.

The clinical reactions noted following administration of the single or combined meningococcal polysaccharide vaccines in Study No. 404 (Table I) are summarized in Table III. The reactions were limited to a mild febrile response in a few persons and to mild local reactions in the majority of subjects given vaccine containing group A antigen. Very few of the subjects showed a reaction following group C vaccine and there was no greater reaction in persons who received combined vaccine than in those given group A vaccine alone.

Multiple doses of vaccine. A small number of persons were given repeat injections of meningococcus A or C vaccine at 0, 1, and 6 months. Blood samples were taken prior to and 1, 3, and 7 months after the initial vaccination. The findings given in Table IV showed the anticipated antibody response to the first dose of vaccine, and there was no further response when an additional one or two doses weje given subsequently.

Vaccine stability. It has been reported that lyophilized meningococcus A polysaccharide vaccine may be unstable on storage at temperatures above freezing (3) (cf. 12–14). Studies were undertaken in which the antibody responses in human subjects to freshly prepared lyophilized meningococcus A or C vaccines were compared with responses to the same vaccines stored at the temperatures for the time periods shown in Table V. There was no detectable decline in immunogenicity of meningococcus A vaccine stored for as long as 1 year at 4° or at −20°, or for meningococcus C vaccine

TABLE II. SERUM BACTERICIDAL ANTIBODY RESPONSES IN HUMAN SUBJECTS IN PENNSYLVANIA MEASURED 2 WEEKS AFTER A SINGLE DOSE OF ONE OF THREE LOTS OF COMBINED MENINGOCOCCUS A-C VACCINE (STUDY NO. 412).

	Antibody response against											
	Meningococcus A						Meningococcus C					
	Initial seropositive			Initial seronegative			Initial seropositive			Initial seronegative		
	≥4× Rise			≥4× Rise			≥4× Rise			≥4× Rise		
Lot no.	No./ total[a]	%	G.M.[b]	No./ total	%	G.M.	No./ total	%	G.M.	No./ total	%	G.M.
			12			<2						<2
C-D560	11/11	100	120	15/15	100	147				25/26	96	50
			9			<2			5			<2
C-D561	4/4	100	147	21/22	95	38	3/4	75	23	22/23	96	57
			12			<2						<2
C-D562	12/13	92	135	12/13	92	61				25/26	96	38

[a] Number showing rise/total number tested.
[b] Geometric mean titer.

TABLE III. CLINICAL FINDINGS AMONG HUMAN SUBJECTS WHO RECEIVED MENINGOCOCCUS A, C, OR COMBINED A-C VACCINE IN STUDIES CARRIED OUT IN PENNSYLVANIA (STUDY NO. 404).

	Meningococcus vaccine[a]—No. positive at time indicated								
	Group A (26 persons)			Group C (25 persons)			Group A-C (26 persons)		
Clinical findings	4 hr	Day 1-3	Day 10	4 hr	Day 1-3	Day 10	4 hr	Day 1-3	Day 10
Local									
Erythema	5	8	0	0	0	0	5	7	0
Induration/swelling	4	4	0	0	0	0	2	5	0
Soreness	13	12	0	2	2	0	14	13	0
Maximum temperature (0)°F									
<99	22	21	22	22	23	23	23	22	19
99-100.9	4	4	0	3	2	0	3	3	1
101.9	0	1	0	0	0	0	0	0	0
Not taken	0	0	4	0	0	2	0	1	6

[a] Same lots of vaccine as Fig. 1.

TABLE IV. SERUM BACTERICIDAL ANTIBODY RESPONSES IN HUMAN SUBJECTS 21-45 YEARS OF AGE FOLLOWING REPEAT INJECTIONS OF MENINGOCOCCAL GROUP A AND C VACCINES (STUDIES NO. 329 AND NO. 281, COSTA RICA).

	Vaccine											
	Meningococcus A (C-A611)						Meningococcus C (C-A258-1)					
	Initial seropositive			Initial seronegative			Initial seropositive			Initial seronegative		
	≥4× Rise			≥4× Rise			≥4× Rise			≥4× Rise		
Antibody following dose	No./ total[a]	%	G.M.[b]	No./ total	%	G.M.	No./ total	%	G.M.	No./ total	%	G.M.
0[c]	—		10	—		<2	—		16	—	—	<2
1	7/7	100	105	2/2	—	23	0/1	—	32	7/9	78	12
2	6/6	100	64	2/2	—	23	0/1	—	16	6/9	67	6
3	6/6	100	72	2/2	—	23	0/1	—	16	6/8	75	6

[a] Number showing rise/total number tested.
[b] Geometric mean titer.
[c] Vaccine given at 0, 1, and 6 months. Serologic tests on bloods taken at 0, 1, 3, and 7 months.

stored at 4° for 1 1/3 years. It is not possible to provide molecular weight data for the meningococcus vaccines used in these studies since the measurement, by K_d determination, was not evolved at the time the studies were done. Retrospective analysis indicates, however, that the K_d value for the A vaccine was about 0.45 and for C about 0.40.

Groups A and C vaccines that were rehydrated with the thimerosal-containing diluent and stored at 4° for 14 days were found to be just as immunogenic as when freshly rehydrated in physiological saline solution. Table VI shows that there was no significant difference either in fourfold or greater antibody titer rises or in geometric mean titers in persons given stored vaccine compared with the fresh product.

Discussion. Meningococcal infections are an important cause for morbidity and mortality, especially during periods of epidemic resurgence. Monovalent polysaccharide vaccines against meningococcus A and C have proved safe and efficacious in trials in man. The vaccines induce far less antibody in young children than in adults (1–10) but meningococcus A vaccine has been proved efficacious in babies 3 months of age or older (9, 10) and meningococcus C in children 2 years or older (5, 10), according to best present evidence.

The administration of these two vaccines in combined form presents a substantial convenience and saving in costs for administration in areas of the world in which there is risk of both group A and C meningococcal diseases. It is of importance, as shown in the present study, that this can be accomplished without reduction in antibody response and

TABLE V. STABILITY ON STORAGE IN LYOPHILIZED FORM OF MENINGOCOCCUS A AND C VACCINES AS REVEALED IN TESTS FOR SERUM BACTERICIDAL ANTIBODY IN ADULT HUMAN SUBJECTS.

Meningococcus vaccine	Storage		Homologous bactericidal antibody responses			
			≥4× Rise		Geometric mean titer	
	Time	Temperature	No./total[a]	%	Prevaccine	Postvaccine[b]
Group A C-D469	Fresh	–	27/29	93	4	82
	1 year	4°	15/15	100	<2	67
	1 year	−20°	13/13	100	<2	75
Group C C-B837	Fresh	–	44/46	96	<2	31
	16 months	4°	44/47	94	<2	98

[a] Number showing rise/total number tested.
[b] Two weeks after vaccination.

TABLE VI. STABILITY OF MENINGOCOCCUS A AND C VACCINE ON REHYDRATION WITH THIMEROSAL PRESERVATIVE SOLUTION AND STORAGE AT 4° FOR 2 WEEKS (STUDIES NO. 388 AND NO. 336).

Meningococcus vaccine	Treatment	Homologous bactericidal antibody responses			
		≥4× Rise		Geometric mean titer	
		No./total[a]	%	Prevaccine	Postvaccine[b]
Group A Lot C-D039 Age range 23 to 61 years Mean 40.6 years	Thimerosal solution 2 weeks	27/27	100	2	94
	Saline solution fresh	23/24	96	4	102
Group C Lot C-A258-1 Age range 22 to 62 years Mean 38.6 years	Thimerosal solution 2 weeks	27/29	93	2	54
	Saline solution fresh	26/28	93	2	55

[a] Number showing rise/total number tested.
[b] One month after vaccination.

without increase in local or systemic reaction.

Serum bactericidal antibodies are presently considered to be the most meaningful indicators for protective immunity (15). The antibody responses to vaccination in adult persons, even in those without detectable antibody initially, are likely based on recall owing to previous experience with meningococcus A and C organisms or with agents bearing related antigens. It is not surprising, therefore, that second or third doses of vaccine given within a short period of time did not elevate the antibody titers above those obtained following the first dose of vaccine.

Lyophilized meningococcus A vaccine is commonly considered to be unstable on storage at 4° (3, 12–14). This was not found for the present material within the time period tested. Meningococcus A vaccine retained full immunogenic potency for 1 year and meningococcus C vaccine retained its potency for $1\frac{1}{3}$ years, the longest time periods tested. Brandt *et al.* (16) reported apparent instability of dried group A vaccine after storage at 4° for 2 years.

The groups A and C vaccines retained their immunogenicity when rehydrated in thimerosal preservative and stored for 2 weeks in the refrigerator. Stability on addition of thimerosal has also been observed by others (17). These are important attributes for the vaccines in their practical routine use.

Summary. Purified group A and C meningococcal polysaccharide vaccines prepared in these laboratories caused remarkably little local or systemic reaction and were highly effective in stimulating serum bactericidal antibody in studies carried out in adults. There was no measurable reduction in immunogenicity or increase in toxic reaction when the two vaccines were given in combined form in a single dose. Maximal antibody response was obtained following a single dose of vaccine and there was no increase in titer when additional doses were given 1 and 6 months later. Both vaccines retained their immunogenic potency on storage at 4° for at least 1 year and there was no reduction in immunogenicity on storage for up to 2 weeks at 4° following rehydration in thimerosal preservative solution.

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