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Clinical and Serological Evaluation of a Meningococcal Polysaccharide Vaccine Groups A, C, Y, and W135 (41306)

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Abstract. The clinical evaluation of a meningococcal polysaccharide vaccine containing groups A, C, Y, and W135 antigens was described. The vaccine was found to be clinically acceptable and a significant antibody response was detected by both a bactericidal assay and a solid-phase radioimmune assay.

Highly purified capsular polysaccharide vaccines prepared against groups A, C, and Y meningococci have been developed (1–4). These vaccines have been shown to be antigenic and free of significant side effects (3–7). Group A, group C, and group A/C combined vaccines have been licensed for distribution and use by the Bureau of Biologics, Food and Drug Administration.

In order to further broaden the potential protection afforded by a meningococcal vaccine, the group W135 polysaccharide was added to the A, C, and Y vaccine. The purpose of this study was to evaluate such a combination vaccine with respect to clinical acceptability and serological response.

Materials and Methods. *Vaccines.* The vaccines used in this study were prepared in the Connaught Laboratories Inc. Research Laboratories using *Neisseria meningitidis* strains A-1, C-11, 6306Y (Slaterus Y), W135 (S-3233). The group A, C, and Y strains were obtained from the Walter Reed Army Institute of Research. The group W135 strain was obtained from Carl Frasch, Ph.D., Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland. The cold phenol method of Gotschlich *et al.* (8) was used to purify the polysaccharides. Molecular sizing of the polysaccharides using Sepharose 4B gel permeation chromatography suggested that the four components were of high molecular weight. The quantities of polysaccharides eluting at or before a partition coefficient of K_d 0.4 were over 60% for group A and group C polysaccharides and over 80% for group Y and group W135 polysaccharides. The individual polysaccharide components A, C, Y, and W135 were tested for pyrogenicity (three rabbits each component) at 0.25 μg/kg. No significant temperature elevations were observed. The final vaccines were tested in rabbits at a concentration of 1.0 μg/kg (0.25 μg each component) and no febrile responses observed. In addition, the vaccine met the general requirements of the Bureau of Biologics, Food and Drug Administration (9), and other existing standards pertinent to the use of vaccines (10).

A placebo consisting of isotonic sodium chloride, lactose (3 mg per dose added as a stabilizer for meningococcal polysaccharides) and 0.01% thimerosal was used in this study (11).

Clinical studies. The study population consisted of healthy adult volunteers, employees, and staff of a university hospital who had given their informed consent. The study population included both men and women, 18 years of age and older. Four lots of vaccine were evaluated in a total of 150 people; one lot was administered to 60 individuals and three lots each administered to 30 individuals. Each person received 0.5 ml (containing 50 μg of each of the four polysaccharides) subcutaneously in the

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TABLE I. CLINICAL OBSERVATIONS, MULTIVALENT MENINGOCOCCAL POLYSACCHARIDE VACCINES: SYSTEMIC REACTIONS

	None	Mild	Moderate
Headache	93.0	5.2	1.8
Malaise	97.5	2.5	0
Chills	97.5	2.5	0
Oral temperature (°F)	96.8 (98.4–99.9)	2.6 (100–101)	0.6 (>101)

Note. Results from 150 individuals, given as percentages.

upper arm in the region of the deltoid muscle. Twenty-five individuals received 0.5 ml of the placebo.

Blood specimens were obtained by venipuncture (antecubital vein) prior to immunization and 4 weeks later. Sera were obtained from one group of individuals one year after vaccination. Sera were collected and stored frozen at −20° until serological testing was conducted.

Clinical observations were made and recorded for each individual at 4-hr intervals until bedtime on the day of immunization, then daily for the next 3 weeks. Oral temperatures were taken for the first 2 days regularly, and continued if the volunteer had any symptoms. Any adverse local or systemic reactions were recorded on individual case report forms.

Serological studies. Bactericidal tests were performed on pre- and postimmunization sera (inactivated at 56° for 30 min) using a procedure developed by the Bureau of Biologics (9).

Antibodies to each group specific polysaccharide were quantitated by a solid-phase radioimmune assay (SPRIA). Details of this method have been submitted for publication. Briefly, the method entails the covalent coupling of purified capsular polysaccharide to poly-L-lysine; this complex is allowed to attach to polystyrene surfaces. Binding of primary antibody to the capsular polysaccharide was detected using ^{125}I-labeled specific anti-human sera. Quantitation of the primary antibody was accomplished using human reference sera (pooled sera from individuals that had received the respective vaccines).

Results. The clinical observations are summarized in Tables I and II. The complaints of individuals receiving the vaccines were related primarily to soreness at the site of injection which lasted no more than 24 to 48 hr after immunization. The clinical response to the vaccines appeared to be quite favorable; no significant systemic reactions were observed.

The serological results are summarized in Tables III and IV. In Table III, a comparison of bactericidal and SPRIA results are presented for three groups of individuals; each group received a different lot of vaccine.

Over 90% of the individuals responded to all four polysaccharide antigens as determined by both serological assays. In Table IV, the responses of two groups of individuals that received the same lot of vaccine (lot 2972) are summarized. Only bactericidal testing was done for these studies, but the results support the previous studies with regard to potency of the respective meningococcal polysaccharide antigens. In addition most of the individuals available one year postimmunization, had high levels of circulating antibodies to the four serogroups of antigens.

TABLE II. CLINICAL OBSERVATIONS, MULTIVALENT MENINGOCOCCAL POLYSACCHARIDE VACCINES: LOCAL REACTIONS

	None	Mild	Moderate
Pain	72	26	2
Tenderness	55	36	8
Diameter	0	<2 in.	≧2 in.
Erythema	95.0	3.8	1.2
Induration	94.4	4.4	1.2

Note. Results from 150 individuals; given as percentages.

TABLE III. SEROLOGIC RESULTS OF MULTIVALENT MENINGOCOCCAL VACCINE CLINICAL TRIALS[a]

	Serogroup A			Serogroup C			Serogroup Y			Serogroup W135		
Vaccine lot	Pre[b]	Post[c]	Conv.[d]	Pre	Post	Conv.	Pre	Post	Conv.	Pre	Post	Conv.
3119												
SPRIA[e]	0.27	5.53		0.20	6.82		0.39	4.44		0.20	3.23	
	±0.003	±0.59	30/30	±0.05	±0.61	30/30	±0.10	±0.68	29/30	±0.30	±0.66	30/30
Bactericidal[f]	<1	7.23		<1	6.07		<1	7.67		<1	8.93	
		±0.37	30/30		±0.44	28/30		±0.37	29/30		±0.24	28/28
3123												
SPRIA	0.43	6.26		0.18	7.04		0.72	4.81		0.20	4.41	
	±0.11	±0.60	25/27	±0.08	±0.62	27/27	±0.22	±0.68	23/25	±0.06	±0.81	26/27
Bactericidal	<1	7.29		<1	7.89		<1	7.62		<1	9.00	
		±0.42	27/28		±0.43	27/27		±0.42	26/26		±0.27	26/26
3130												
SPRIA	0.22	5.38		0.13	7.16		0.41	5.50		0.28	3.69	
	±0.05	±0.57	30/30	±0.05	±0.61	30/30	±0.17	±0.67	30/30	±0.16	±0.65	28/28
Bactericidal	<1	8.77		<1	6.86		<1	6.79		<1	9.03	
		±0.30	30/30		±0.59	26/29		±0.45	28/28		±0.33	28/29

[a] Solid-phase radioimmune assay (SPRIA) and bactericidal assay.
[b] Prebleed serum.
[c] 4-week postbleed serum.
[d] Conversions, number positive/number tested; SPRIA positive two-fold or greater increase, bactericidal positive four-fold or greater increase between pre- and postsera antibody.
[e] μg/ml antibody, mean ± SE.
[f] $\log_2$ titer, mean ± SE.

Discussion. This study indicates that the combined meningococcal polysaccharide vaccine, groups A, C, Y, and W135, is safe and clinically acceptable. The addition of the W135 polysaccharide to the vaccine did not alter the previous observations that the purified capsular polysaccharides of the meningococcus have virtually no adverse effect on recipients of the vaccine.

The serological conversions to the groups A, C, and Y were essentially the same as observed previously (3, 4) further supporting the conclusion of an independent immune response to each component of the vaccine. The response to the W135 polysaccharide was of an equal magnitude compared to the responses to groups A, C, and Y. Therefore, following the correlation

TABLE IV. BACTERICIDAL TITERS[a] OF SERA FROM MENINGOCOCCAL VACCINE TRIALS (LOT NO. 2972)

	Serogroup A			Serogroup C			Serogroup Y			Serogroup W135		
Study No.	Pre[b]	Post[c]	Conv.[d]	Pre	Post	Conv.	Pre	Post	Conv.	Pre	Post	Conv.
1441-1												
4 week	<1	8.19		<1	6.95		<1	7.67		<1	7.38	
		±0.50	22/23		±0.50	21/22		±0.38	23/23		±0.56	19/21
1 year		6.62			5.23			5.46			6.54	
		±0.98	10/13		±0.77	12/13		±0.82	10/13		±0.67	12/13
1441-2												
4 week	<1	8.03		<1	6.90		<1	6.90		<1	6.77	
		±0.50	29/30		±0.66	25/28		±0.49	26/29		±0.42	24/26

[a] $\log_2$ titer.
[b] Prebleed serum.
[c] 4-week or 1-year postbleed serum, mean ± SE.
[d] Positive responses/number tested; bactericidal positive fourfold or greater increase between pre- and postsera antibody.

that has been made between the presence of bactericidal antibody and resistance of meningococcal disease (12), the groups A, C, Y, and W135 components should offer an effective vaccine.

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