

## PROTECTION AGAINST GROUP B MENINGOCOCCAL DISEASE

### III. Immunogenicity of Serotype 2 Vaccines and Specificity of Protection in a Guinea Pig Model\*

BY CARL E. FRASCH AND JOAN D. ROBBINS

(From the Bureau of Biologics, Bethesda, Maryland 20014)

Meningococcal disease remains a worldwide problem (1). Approximately 2,000 sporadic cases occur yearly in the United States, and outbreaks have recently occurred in other countries (2-4). Group B *Neisseria meningitidis* is currently responsible for most meningococcal infections in the United States (5), England (6), and Europe. Though effective polysaccharide vaccines for meningococcal groups A and C have been licensed, an immunogenic group B polysaccharide vaccine is not available (7). This has necessitated examination of other cell components against which protective antibodies may be elicited (8, 9).

Reports of increased susceptibility to Neisserial infection in individuals lacking one of the higher complement components (10), but not in those with chronic neutropenia (11), indicate that bactericidal antibody is required for protection against meningococcal disease (12). Much of the anti-group B bactericidal antibody is directed against protein serotype antigens (STA)<sup>1</sup> (13, 14). A synergistic protective effect exists between the group B polysaccharide and serotype antibodies (15), suggesting that combined vaccines may afford the best protection.

Group B meningococci have been subdivided into a number of serotypes based on the presence of immunologically distinct STA (13, 16). The major outer membrane proteins of each of the serotypes probably contain the antigenic moiety responsible for type specificity (16). The serotype 2 antigen was chosen for initial vaccine trials because this serotype is frequently associated with meningococcal disease (6, 17, 18).

These studies describe the methods for preparation of immunogenic type 2 protein vaccines, and their evaluation in a guinea pig chamber implant model (19). The vaccines were immunogenic and protected against infection by the

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<sup>1</sup> Abbreviations used in this paper: DOC, deoxycholate; EBC-720, Emulphogene BC-720; ELISA, enzyme-linked immunosorbant assay; HBSS-BSA, Hanks' balanced salt solution containing 0.1% bovine serum albumin; KDO, 2-keto-deoxyoctonate; LAL, Limulus amoebocyte lysate; LPS, lipopolysaccharide; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; STA, serotype antigens; TSB, tryptic soy broth; Tx-100, Triton X-100.

homologous meningococcal serotype regardless of serogroup. These studies elaborate upon findings presented in a preliminary report (20).

### Materials and Methods

*Strains and Growth Conditions.* The group B, type 2 meningococcal strains used were M986 and a noncapsular variant of M986 designated M986-NCV-1. Meningococcal strains used for guinea pig challenge, growth conditions, and method of challenge have been described (19). For vaccine preparation the strains were grown either in 12 2.8L baffled Fernbach flasks (Bellco Glass, Inc., Vineland, N. J.) containing 1.4 liters tryptic soy broth (TSB, Difco Laboratories, Detroit, Mich.) each, or in a 50-liters fermentor. In the first method the cultures were incubated overnight at 36°C at 125 rpm on a gyrotory shaker, then harvested by centrifugation. For the fermentor procedure, the NCV-1 strain was grown for 6 h in two Fernbach flasks (1.4 liters TSB each), then inoculated to 45 liters of TSB in the fermentor. The organisms were grown overnight at 37°C with 40 liters air/min by sparge and agitation of 200 rpm. The cells were harvested from the fermentor with a Sharples continuous flow centrifuge. (Sharples-Stokes Div., Pennwalt Corp., Warminster, Pa.)

*Preparation of Vaccines.* The starting material for vaccine preparation was the serotype 2 STA, purified from 0.2 M LiCl-0.1 M Na Acetate, pH 5.8, extracts by ultracentrifugation as previously described (8). Vaccines were prepared by three different procedures: (a) The STA was suspended to a protein concentration of 1-2 mg/ml in 0.01 M Tris (hydroxy methyl) amino-methane-HCl 0.01 M (ethylenedinitrilo)-tetraacetic acid buffer pH 8.5 (Tris-EDTA) and treated with 5% (vol/vol) Triton X-100 (TX-100) for 30 min at room temperature. The serotype protein was pelleted by centrifugation at 100,000 *g* for 4 h, resuspended in Tris-EDTA buffer containing 2% (vol/vol) TX-100 and reultracentrifuged. The pellet was resuspended in 0.15 M sodium chloride and the protein precipitated, with 4 vol of 95% ethanol at -20°C overnight. The antigen was then treated with acetone at room temperature for 1 h to effect sterilization. The pellet was washed with ethanol to remove acetone. The vaccine was prepared by suspending the pellet in sterile 0.15 M sodium chloride at 1 mg/ml protein. (b) The initial solubilization and centrifugation procedures were exactly as above, except for substitution of Emulphogene BC-720 (EBC-720, GAF Corp., New York) in place of TX-100. After the initial detergent treatment, the antigen preparation was ultracentrifuged twice in 2% detergent. The pellet was dissolved in the Tris-EDTA buffer containing 0.5 M sodium chloride and 5% (vol/vol) EBC-720, prefiltered, then passed through an 0.22  $\mu$ m membrane filter (Millipore Corp., Bedford, Mass.). The protein was recovered by addition of 4 vol of 95% ethanol previously passed through an 0.2  $\mu$ m Nucleopore filter (Nucleopore Corp., Pleasanton, Calif.). The pellet was washed twice with filtered ethanol and resuspended as final vaccine to approximately 1 mg protein/ml in sterile 0.15 M sodium chloride. (c) The STA was treated with 1% (wt/vol) deoxycholate (DOC, Sigma Chemical Co., St. Louis, Mo.) in 1 mM Tris-HCl, 10 mM EDTA pH 8.5 for 30 min at room temperature. The serotype protein was then pelleted by centrifugation at 100,000 *g* for 4 h. The pellet was washed once with 1 mM Tris-EDTA buffer containing 0.1% DOC. Alternatively, the STA was solubilized in DOC, then separated by gel filtration on Sephadex G150 (Pharmacia Fine Chemicals, Piscataway, N. J.) in the Tris-EDTA buffer containing 0.5% DOC. The STA eluted at the void volume and was recovered by ethanol precipitation (80% vol/vol). The protein antigen was resuspended in Tris-EDTA buffer containing 2% DOC and heated for 30 min in a 56°C water bath, then sterile filtered. The antigen was precipitated and washed as described for the EBC-720 vaccines.

*Chemical and Pyrogenic Analysis of Vaccines.* Protein was measured by method of Lowry et al. (21) with bovine serum albumin (fraction V) as a standard. Protein composition was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8). Lipopolysaccharide (LPS) was measured by 2-keto-deoxyoctonate (KDO) determination (22) with KDO (Sigma Chemical Co.) as a standard. Amino acid analysis was determined after hydrolysis at 115°C in 6 N hydrochloric acid for 22 h. Sialic acid content was estimated on a Beckman amino acid analyzer (Beckman Instruments, Inc., Cedar Grove, N. J.) after mild acid hydrolysis (23). Nucleic acids were determined by examination for ribose by the method of Boykins and Liu (manuscript in preparation).

The vaccines were tested for endotoxin content by the *Limulus* amoebocyte lysate gelatin test (LAL) using a *Klebsiella* LPS reference standard (24, 25). In some experiments meningococcal LPS prepared from strain M986 by hot phenol-water (8) was used as a standard. Selected vaccine lots were also examined for rabbit pyrogenicity with the standard U. S. Pharmacopoeia rabbit test and for a dermal Shwartzman reaction (26).

*Immunization and Challenge of Guinea Pigs.* The antibody response to the different vaccines was evaluated in 250–500 g female NIH Hartley strain guinea pigs. The animals were injected intramuscularly with different doses of vaccine in 0.1 ml saline. This was usually followed by a booster given 2–3 wk later. The protective effects of immunization were examined using the stainless steel chamber implant model of Arko and Frasch and Robbins (27, 19). The guinea pigs were challenged 10–14 days after implantation with approximately  $5 \times 10^6$  log phase meningococci injected into the spring chamber. The animals were sampled and bled as before (19).

*Quantitation of Serotype Antibody.* Enzyme-linked immunosorbant assay (ELISA) was used for quantitation of serotype antibodies (28). Serum IgG from a guinea pig immunized with an EBC-720 vaccine was prepared by ion exchange chromatography on Whatman DE-52 (Whatman Chemicals Div. W & R Balston, Maidstone, Kent, England) in 0.02 M Tris-HCl pH 8.2. Serum dialyzed against this buffer was applied to the column. The IgG eluting in the fall-through volume was quantitated assuming an extinction coefficient of 1.4 at 280 nm. Purity of the IgG was evaluated by Microzone electrophoresis on acetate strips (Beckman Instruments, Inc., Palo Alto, Calif.). We labeled 150  $\mu$ g of this IgG fraction with  $^{125}$ I using the chloramine-T method of Greenwood et al. (29). The percentage of IgG bound to type 2 STA immobilized on the surface of  $10 \times 75$ -mm polystyrene tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) was then determined at five different IgG concentrations, from which the concentration of type 2 antibody/ml was calculated. A standard curve for each ELISA was plotted, relating absorbance at 400 nm at 100 min to nanogram antibody in the IgG standard.

*Bactericidal Assay.* A modification of the microbactericidal assay (13) was used for measurement of bactericidal antibody in selected guinea pig sera. The sera were titrated in flat bottom microtissue culture trays (Falcon Plastics, model 3040), using Hanks' balanced salt solution containing 0.1% bovine serum albumin (HBSS-BSA) as diluent. The meningococci were grown to log phase (4–5 h) on the surface of brain-heart infusion plates, then diluted to 78% transmission at 540 nm in phosphate-buffered saline (PBS) pH 7.4. The organisms were further diluted to yield approximately 2,000 organisms/ml, the final dilution being in HBSS-BSA. The source of complement was 4-wk-old baby rabbit serum (type 3, Pel-Freez Farms, Inc., Rogers, Ark.) diluted 1:2. The trays were incubated at 37°C for 30 min, approximately 0.2 ml of TSB (Difco Laboratories) containing 1.2% Noble agar (Difco Laboratories) at 45°C was added to each well, and further incubated 24 h at 37°C, then read. The endpoint was the highest serum dilution causing 50% or greater reduction in viable count.

## Results

*Preparation and Analysis of Protein Vaccines.* The STA complex contains similar amounts of protein and LPS (16). The type 2 STA was treated with detergents to preferentially solubilize the toxic LPS component. While TX-100 nonspecifically solubilized both the LPS and protein components, EBC-720 preferentially solubilized the LPS. The major 41,000 dalton protein component of the outer membrane remained within the high molecular weight complexes after treatment of the STA with 5% EBC-720 in the low ionic strength Tris-EDTA buffer. Upon removal of detergent, most vaccine preparations became particulate.

Various methods were investigated for sterilizing the STA vaccines without altering their immunologic properties. In preliminary experiments, small vaccine lots prepared using TX-100 were treated with acetone, but this treatment did not sterilize all lots, since acetone does not kill bacterial spores (30). The vaccines were effectively sterilized by membrane filtration when the

detergent-treated ultracentrifugation pellet was resuspended in 5% EBC-720 in Tris-EDTA buffer containing 0.5 M sodium chloride.

EBC-720, TX-100, and DOC vaccines, prepared from different batches of M986, were compared with an untreated STA by SDS-PAGE (Fig. 1). Solubility of the different proteins of the STA complex varied with the detergent used. DOC-treated vaccines generally contained primarily the major 41,000 dalton protein. The TX-100 and EBC-720 vaccines were approximately 86% protein, while the DOC prepared vaccines contained up to 97% protein (Table I). LPS was the only other significant component detected in the vaccines. Group B polysaccharide was not detected and no nucleic acids were detected.

The endotoxin (i.e. LPS) content of the vaccines was estimated by KDO content, the LAL gelation test, and by rabbit pyrogenicity (Table I). The amount of endotoxin detected by LAL was considerably less (10- to 200-fold) than that calculated from the KDO content. A *Klebsiella* LPS was regularly used as a standard for LAL. The sensitivity of the assay was the same using a meningococcal LPS standard. The different results with the two methods are most likely explained by the particulate nature of the vaccines, since LAL probably detects only the endotoxin on the particle surface. Some antigen preparations (not shown in tables) remained nonparticulate after detergent treatment, and in these preparations the LAL test gave endotoxin values comparable to those obtained by KDO determinations. The rabbit pyrogenicity determinations agreed very well with the LAL results, indicating that the rabbits also respond primarily to the surface endotoxin.

The DOC and EBC-720 vaccines were further characterized by amino acid analysis and compared to that of the untreated STA (Table II). The glucosamine content varied in direct proportion to the amount of LPS present, and therefore can be used to estimate the amount of LPS in vaccines. Phenol-water purified LPS of the vaccine strain contained 3.3% glucosamine (C. E. Frasch and J. D. Robbins, unpublished data).

*Quantitation of Antibody Response.* Due to the amount of serum available and the protein nature of the antigen, antibody measurements were not done by the quantitative precipitin method. For quantitation of the type 2 STA antibody, an IgG fraction of the standard serum was prepared by DEAE chromatography and compared to the unfractionated serum for serotype reactivity by ELISA (Fig. 2 A). Most of the type specific antibody was present in the IgG fraction. Comparison of labeled and unlabeled IgG indicated that the labeling did not alter its serological reactivity. Finally, the percent of total IgG reacting with an excess of type 2 STA was determined. Over a series of twofold dilutions of the IgG fraction from 1/50 to 1/800, the STA bound  $2.94 \pm 0.05$  percent of total IgG. The IgG standard was run with each ELISA experiment. The standard curve was linear between approximately 1 and 100 ng specific antibody (Fig. 2 B).

*Vaccine-Induced Antibody Response.* In all experiments, the vaccines were injected intramuscularly into the animal's thigh without adjuvants. First, the relative immunogenicity of untreated STA was compared to the EBC-720 and DOC vaccines (Fig. 3). Each vaccine was administered in two doses 2 wk apart, 200 and 100  $\mu$ g being given in the first and second doses, respectively. The

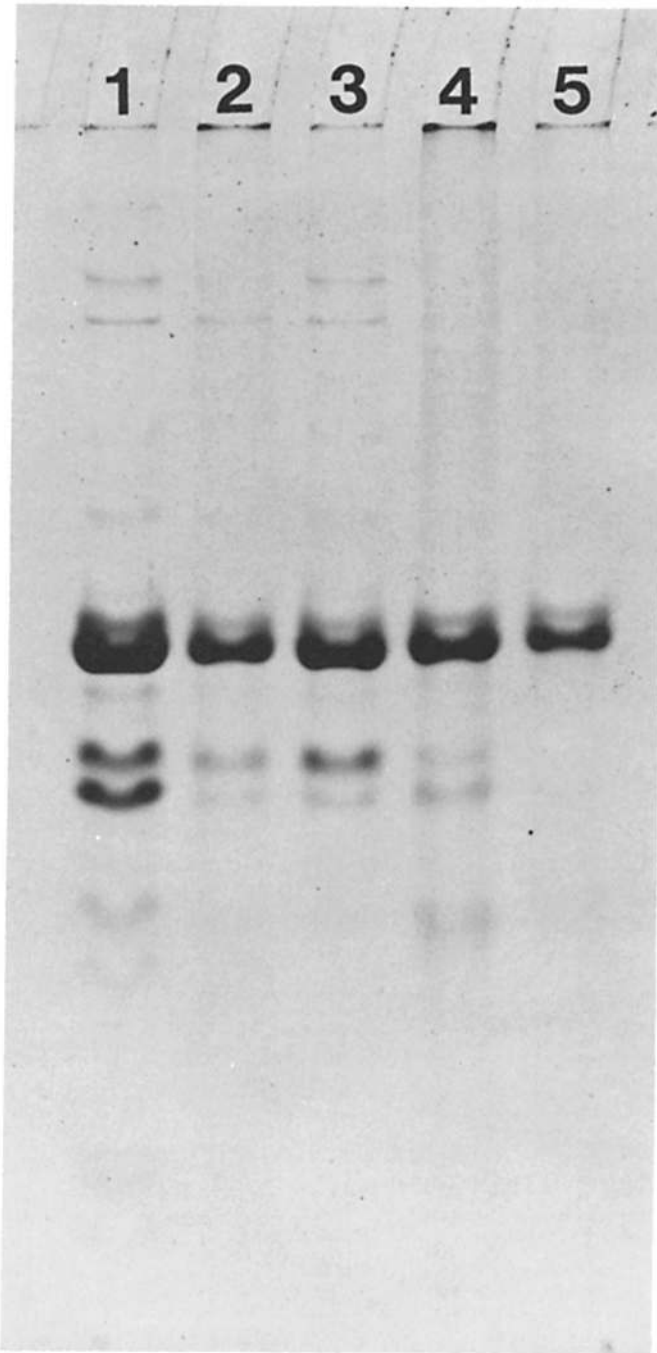


FIG. 1. Comparison of the SDS-PAGE patterns of vaccines prepared by detergent treatment of type 2 STA. The gel patterns: (1) M986-NCV-1 STA; (2) EBC-720 vaccine lot E-05; (3) TX-100 vaccine lot V-3; (4) DOC vaccine lot VMF-1, prepared by ultracentrifugation; and (5) a DOC vaccine prepared by gel filtration on Sephadex G150.

TABLE I  
*Comparison of Serotype 2 Vaccines Prepared from M986 and M986-NCV-1 with Different Detergents*

Vaccine lot	Detergent used	Protein	LPS	$\mu\text{g LPS/mg Protein}$		MPD‡
				TBA*	LAL	
		$\mu\text{g/mg}$	$\mu\text{g/mg}$			$\mu\text{g protein}$
STA	—	425	301	708	—§	—
V-1	TX-100	840	160	190	—	—
V-3	TX-100	860	140	163	10	0.9
DOC-1	DOC	920	80	87	0.2	5.6
D-1	DOC	970	30	31	—	—
VMF-1	DOC	920	80	87	1.8	3.4
E-1	EBC-720	870	130	75	5.0	—
E-2	EBC-720	880	120	136	0.6	—
E-3	EBC-720	860	140	158	—	—

\* TBA, Thiobarbituric acid assay.

‡ MPD, Minimum pyrogenic dose in New Zealand white rabbits represented as  $\mu\text{g protein/kg}$  rabbit.

§ Not done.

TABLE II  
*Amino Acid and Amino Sugar Composition of the Untreated Serotype 2 Antigen and Type 2 Vaccines*

Component	STA	EBC-720 Vaccine	DOC Vaccine
	<i>moles %*</i>		
Lysine	8.53	10.28	8.02
Histidine	2.51	2.79	2.17
Arginine	6.17	6.51	5.31
Aspartic acid	8.33	9.58	10.01
Threonine	4.29	4.74	5.10
Serine	6.15	6.66	6.93
Glutamic acid	8.32	9.37	9.44
Proline	1.86	1.86	2.37
Glycine	10.50	10.50	11.59
Alanine	8.35	9.64	11.59
Cysteine	0.00	0.00	0.00
Valine	6.90	8.13	8.76
Methionine	1.09	0.95	1.09
Isoleucine	2.81	2.94	3.08
Leucine	4.34	4.81	4.94
Tyrosine	4.59	5.59	4.63
Phenylalanine	4.07	4.56	4.27
Glucosamine	4.29	1.09	0.69

\* Amino acid composition not corrected for degradation.

antibody response was measured 2 and approximately 5 wk after primary immunization. Preimmunization sera contained  $<0.1 \mu\text{g/ml}$  STA antibody. The peak mean antibody concentrations were 88, 28, and  $13 \mu\text{g/ml}$  for the STA, EBC-720, and DOC vaccines, respectively. The untreated STA proved toxic to

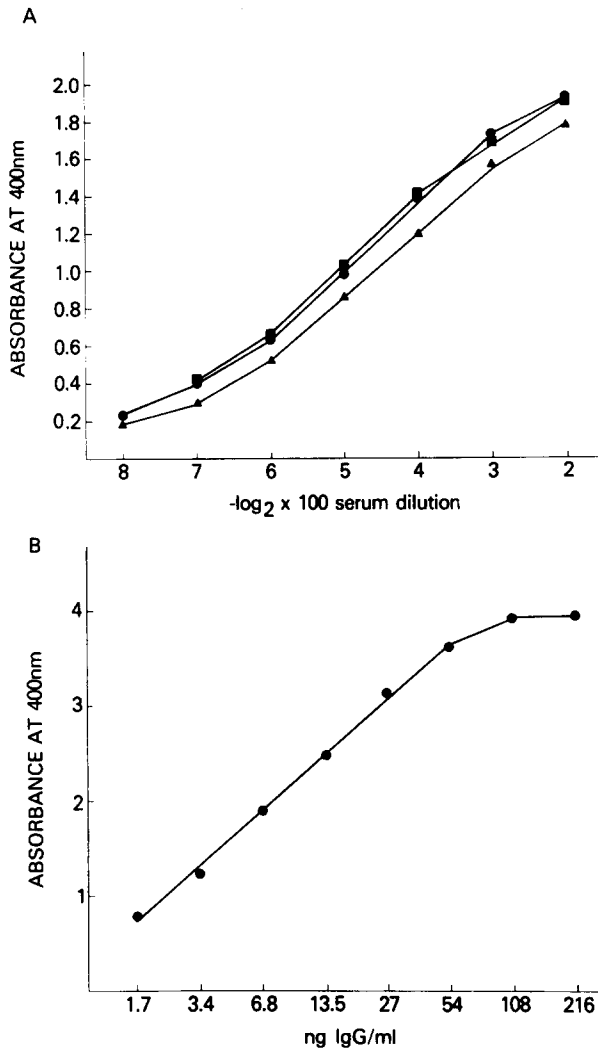


FIG. 2. Quantitation of specific antibody in an IgG fraction of an anti-serotype 2 guinea pig serum. (A) Comparison of binding curves for the DEAE prepared IgG from serum 9-P (●) with the whole 9-P serum (■) and another guinea pig immune serum, 7-G (▲). (B) A standard curve using the IgG fraction.

the animals, but animals injected with the detergent treated antigens showed no ill effects from either injection.

To examine the primary immune response and determine whether a booster response would occur, animals were given a 50  $\mu$ g dose of DOC vaccine, Lot VMF-1 or VMF-2, and a second dose 8 wk later. Sera were obtained at 1-2-wk intervals throughout the experiment (Fig. 4). The primary immunization resulted in a slight antibody rise of approximately 0.5  $\mu$ g antibody/ml (see Table III). The second dose of vaccine caused a rapid 6- to 20-fold rise in antibody which persisted at elevated levels. The peak antibody response was comparable to that observed when vaccine doses were presented 2-3 wk apart.

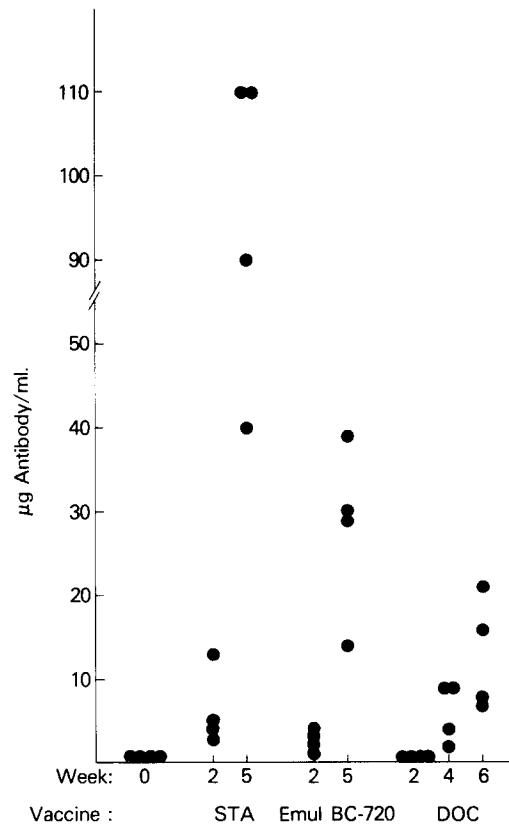


FIG. 3. Serotype 2 antibody response measured by ELISA after immunization with type 2 STA, TX-100 prepared vaccine, and DOC prepared vaccine. The vaccines were given in doses of 200  $\mu\text{g}$  at wk 0 and 100  $\mu\text{g}$  at wk 2.

The various vaccine preparations and doses used in the protection studies are listed in Table III. When administered in two doses, most vaccines elicited a mean of  $>25$   $\mu\text{g}$  antibody/ml serum 5 wk after primary immunization. There was no correlation between LPS content of vaccines and antibody response. However, since all vaccines tested contained LPS to varying degrees, and the serotype 2 antigen used in ELISA was a lipoprotein-lipoplysaccharide complex, it was necessary to determine the relative antibody response to the individual protein and LPS components (Table IV). No LPS antibody was detected. Almost identical  $\text{OD}_{400}$  values were obtained with the STA and a relatively LPS-free protein antigen (vaccine lot E-02).

To determine the most effective dosage, a TX-100 vaccine was used in a dose-response experiment (Fig. 5). Groups of animals received two equal doses of vaccine 2 wk apart. The dose range was between 25 and 200  $\mu\text{g}$  protein. As a control, one set of animals received 100  $\mu\text{g}$  of group B polysaccharide attached to methylated bovine serum albumin for its adjuvant effect. For both primary and secondary immunizations, the 25  $\mu\text{g}$  dose elicited approximately as much antibody as did the higher dosages. In animals given the polysaccharide vaccine, no anti-capsular or serotype antibodies were detected by ELISA.



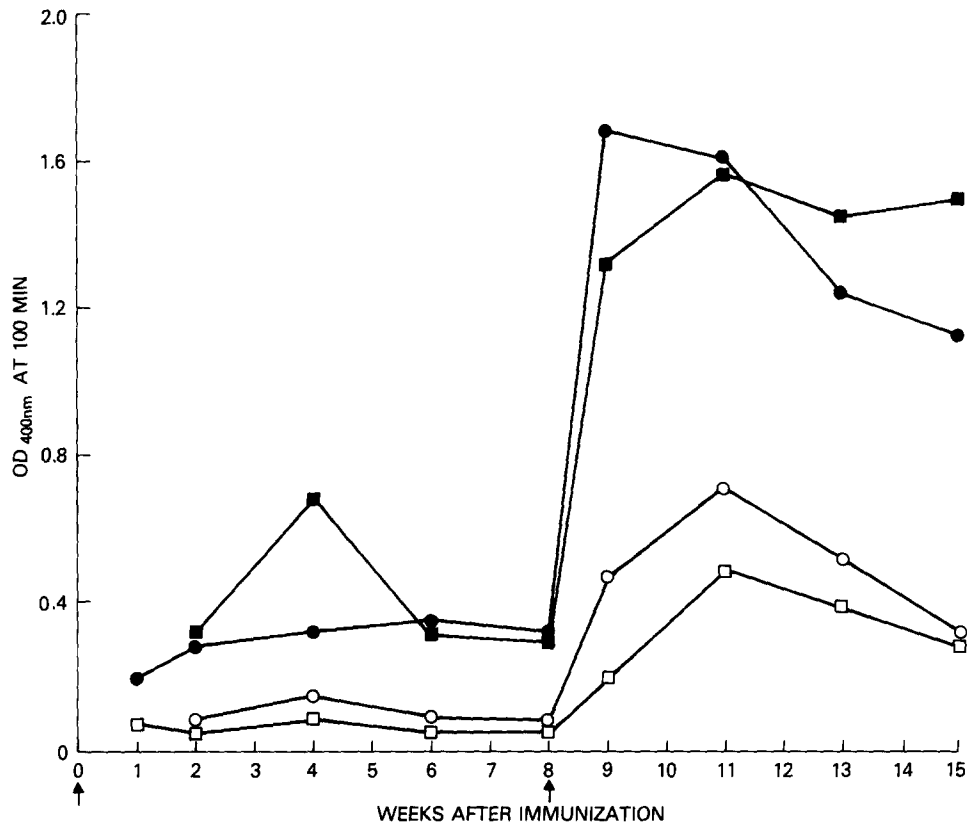


FIG. 4. Anamnestic response of guinea pigs to a second immunization with serotype 2 vaccine. Animals were immunized with 50- $\mu$ g doses of vaccine VMF-1 (solid symbols) or vaccine VMF-2 (open symbols). Arrows denote when vaccines were administered. Antibody against the STA was measured by ELISA.

Since protection against meningococcal disease has been correlated with presence of bactericidal antibodies (10, 11, 28) an *in vitro* bactericidal assay and ELISA were performed on sera obtained at 2-3 wk intervals after immunization with two of the vaccines (Fig. 6). Both vaccines elicited peak bactericidal titers of 1:1,600 to 1:6,400. The antibody response curves obtained by bactericidal assay and by ELISA were similar, indicating that the same antibody was probably being measured in both assays.

*Vaccine-Induced Protection.* To study resistance to infection, we used a guinea pig model in which subcutaneous spring chambers can be infected with small numbers of meningococci. Nonvaccinated animals eliminate their infections in 12-14 days and clearance of infection is associated with appearance of circulating antibody (19).

Experiments were designed to determine whether low antibody levels induced by a single immunization would protect against homologous meningococcal challenge, and whether a subprotective immunization would have demonstrable effects upon the severity of infection (Table V). Animals were challenged with  $5 \times 10^5$  bacteria of strain S-946 3 wk after immunization. 50% of the animals

TABLE III  
Antibody Response in Guinea Pigs to Different Serotype 2 Vaccine Preparations

Animal set	No. animals	Vaccine lot	Detergent used	Dose		$\mu\text{g}$ LPS per dose*	Mean $\mu\text{g}/\text{ml}$ antibody $\ddagger$	Range
				1°	2°			
<i><math>\mu\text{g}</math> protein</i>								
1	4	STA	None	200	100	22	87.5	40-110
2	4	E-1	EBC-720	200	100	16	28.0	14-39
33	4	E-4	EBC-720	50	50	7	25.5	11-59
8	4	T-1	TX-100	50	50	8	29.8	6-50
9	4	T-1	TX-100	25	25	4	32.0	14-54
24	4	V-3	TX-100	50	50	7	57.0	4-240
14	4	D-1	DOC	100	100	3	13.0	7-21
25	4	D-3	DOC	50	50	5	39.5	9-92
32	4	D-4	DOC	50	50	10	45.8	13.5-65
27	4	VMF-1	DOC	50	—	4	0.8	0.5-1.6
28	4	VMF-2	DOC	50	—	2	0.3	0.2-0.4

\* LPS determined by analysis of KDO content of vaccine by TBA assay.

$\ddagger$  Antibody determinations were done by ELISA on sera taken 5 wk after first immunization; the mean preimmunization value was  $<0.1 \mu\text{g}/\text{ml}$ .

TABLE IV  
Specificity of Antibody Response Measured by ELISA in Guinea Pigs 5 Wk after Immunization with Serotype 2 Vaccines

Animal set	Vaccine lot	Dose	$\mu\text{g}$ LPS per dose	Mean OD <sub>400</sub> value with antigen*		
				STA	STA-Protein	LPS
<i><math>\mu\text{g}</math> protein</i>						
1	STA	150	22	3.56	1.97	0.02
8	T-1	50	8	2.37	2.16	0.02
24	V-3	50	7	2.26	1.96	0.03
14	D-1	100	3	1.86	1.74	0.02
25	D-3	50	5	1.87	1.44	0.02
32	D-4	50	10	1.63	1.50	0.01

\* The OD<sub>400</sub> was calculated at 100 min for a serum dilution of 1/400.

who received 25 or 50  $\mu\text{g}$  of serotype vaccine resisted infection. Even in animals that received only 1  $\mu\text{g}$  of protein, the severity of infection was significantly altered as measured by numbers of bacteria in the chambers and duration of infection.

The degree and specificity of protection were examined after two-dose immunization (Table VI), because a severalfold greater antibody response occurred with two doses. Approximately 90% of animals were protected against serotype 2 strains regardless of serogroup, but 50% of animals challenged with heterologous group B strains were also resistant. Resistance to infection persisted for at least 4 mo after booster immunization. Those animals that became infected with type 2 organisms had only low level infections (ca 2,000 organisms/milliliter on day 2) that did not persist beyond day 4. Animals that

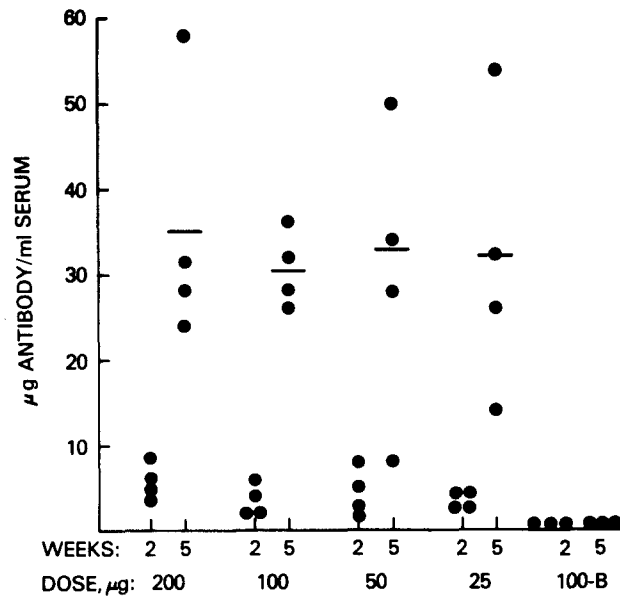


FIG. 5. Dose-response in guinea pigs to a TX-100 vaccine. Booster injections were given 2 wk after primary immunization. One set of animals received two doses of 100  $\mu\text{g}$  of group B meningococcal polysaccharide conjugated to methylated bovine serum albumin.

received group B polysaccharide were not protected, which correlates with failure to detect antibody in these animals.

### Discussion

Serotype 2 was chosen for initial vaccine development because of its association with meningococcal disease caused by groups B, C, Y, and W135 (17, 31), and its association with several meningococcal outbreaks, including the recent group C São Paulo epidemic (6, 18).

It is important to standardize the culture conditions because the protein and LPS composition of the outer membrane may be altered by changes in growth conditions (16). The vaccines used in the present study were prepared by detergent treatment of the STA complex. For selective removal of LPS from the complex, EBC-720 proved best, while TX-100 was the least selective. Analysis of vaccines for LPS by KDO content always yielded higher endotoxin values than did either the Limulus lysate test or rabbit pyrogenicity. This difference is probably due to the particulate nature of the vaccine.

In dose-response experiments, a wide range of effective dosage was evident. As little as 1  $\mu\text{g}$  antigen stimulated detectable antibody levels. A single 50  $\mu\text{g}$  injection of vaccine elicited a peak antibody response at 3-4 wk of about 0.5  $\mu\text{g}/\text{ml}$  serum though it was insufficient to protect more than 50% of animals. A second immunization 2-8 wk later resulted in an anamnestic response with mean peak antibody levels of 25-50  $\mu\text{g}/\text{ml}$  serum. Thus, a two-dose immunization schedule seems necessary to provide the best protection against challenge.

All vaccine lots elicited a bactericidal antibody response, which correlated

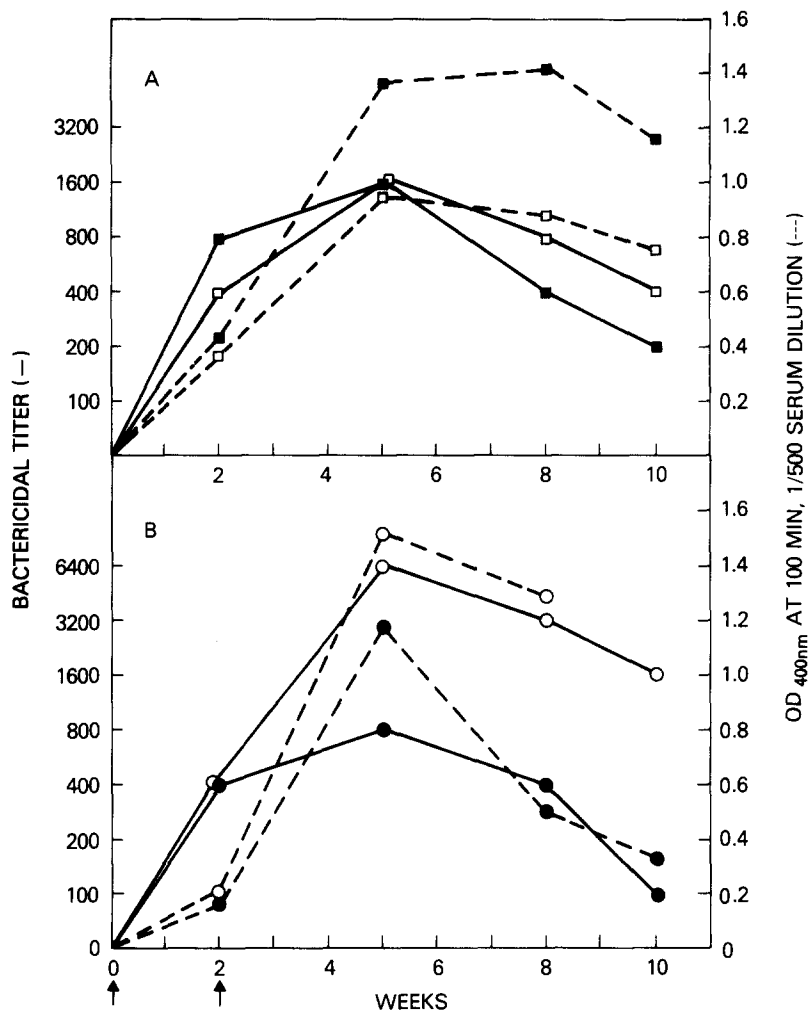


FIG. 6. Comparison of bactericidal titers and serotype 2 antibody response measured by ELISA. (A) Animals (set 33) were immunized with two 50- $\mu$ g doses of EBC-720 vaccine lot E-4. (B) Animals (set 32) were immunized with two 50- $\mu$ g doses of DOC vaccine lot D-4. Arrows denote when vaccines were administered.

closely with the ELISA values. Some lots of vaccine stimulated greater amounts of antibody than others. This variation in antibody response was not attributable to adjuvant effects of the endotoxin, as there was no correlation with LPS content of vaccine, nor was there a detectable antibody response to the LPS after immunization. The differences in antibody response to the different lots may be due to yet unknown differences in their physical-chemical structure resulting from detergent treatment.

Guinea pigs immunized with the serotype 2 protein were resistant to challenge with type 2 organisms, regardless of their group specific polysaccharide. Some protection against heterologous group B strains was observed. This protection may be related to the cross-reactivity of the different serotype antigens seen in an antigen binding assay (16).

TABLE V  
*Effects of a Single Dose Immunization upon a Subsequent Meningococcal Infection (Strain S946) 3 Wk later*

Animal set	Vaccine lot	Dose	Mean serum bactericidal titer*	Animals infected/injected on day (average organisms/milliliter)			
				2	5	7	11
		<i>µg protein</i>					
51	D-05	50	176	2/4 (4 × 10 <sup>4</sup> )	2/4 (4 × 10 <sup>4</sup> )	1/4 (6 × 10 <sup>5</sup> )	0/4
52		10	128	4/4 (6 × 10 <sup>4</sup> )	4/4 (1 × 10 <sup>4</sup> )	4/4 (8 × 10 <sup>5</sup> )	1/4 (4 × 10 <sup>5</sup> )
53		1	40	4/4 (4 × 10 <sup>5</sup> )	4/4 (1 × 10 <sup>5</sup> )	4/4 (5 × 10 <sup>5</sup> )	1/4 (1 × 10 <sup>5</sup> )
55	E-02	50	56	2/4 (4 × 10 <sup>4</sup> )	2/4 (7 × 10 <sup>4</sup> )	1/4 (1 × 10 <sup>5</sup> )	0/4
56		25	81	2/4 (1 × 10 <sup>5</sup> )	0/4	0/4	0/4
57		10	22	3/4 (8 × 10 <sup>5</sup> )	2/4 (4 × 10 <sup>4</sup> )	2/4 (2 × 10 <sup>5</sup> )	1/4 (2 × 10 <sup>5</sup> )
58		1	22	3/4 (7 × 10 <sup>5</sup> )	3/4 (2 × 10 <sup>5</sup> )	3/4 (2 × 10 <sup>5</sup> )	0/4
54	A/C‡	50	<2	4/4 (3 × 10 <sup>7</sup> )	4/4 (9 × 10 <sup>6</sup> )	4/4 (7 × 10 <sup>6</sup> )	2/4 (7 × 10 <sup>5</sup> )

\* The bactericidal titer was determined just before challenge using the group B type 2 strain B16B6.

‡ A/C Meningococcal polysaccharide vaccine, groups A and C combined.

TABLE VI  
*Resistance to Meningococcal Infection 3-17 Wk after Immunization with Two Doses of Serotype 2 Vaccine*

Animal set	Vaccine lot	Dose	Weeks after second immunization	Challenge with strain*				
				S-946 (B,2)‡	138I (C,2)	S-1975 (Y,2)	M1080 (B,1)	M982 (B,9)
		<i>µg protein</i>						
2	E-1	200	3	1/4§	—	—	1/3	—
7	T-1	100	3	0/4	0/4	—	—	—
8	T-1	50	3	0/4	1/4	—	—	—
9	T-1	25	3	0/4	1/4	—	—	—
3	D-2	200	3	0/4	—	—	2/4	—
24	V-3	50	8	1/4	—	0/4	—	—
33	E-4	50	9	0/4	—	—	—	2/4
25	D-3	50	8	1/3	0/3	—	—	—
32	D-4	50	9	0/3	—	—	2/3	—
27	VMF-1	50	17	0/4	0/4	1/4	—	—
Combination	type 2 vaccines	—	—	3/38(92)	2/19(89)	1/8(88)	5/10(50)	2/4(50)
10	B polysaccharide	100	3	3/3	—	—	—	—

\* Challenge dose approximately 3 × 10<sup>8</sup> Meningococci.

‡ (Serogroup, serotype).

§ Infected/challenged.

|| Percent of animals protected.

Meningococcal disease as well as carriage induce high levels of serotype antibody in both adults and children (28, 32). Presence of elevated serotype antibody levels in convalescent sera of infants after meningococcal disease (28) indicated that the STA was immunogenic in children under 1 yr of age. An STA vaccine may therefore protect very young children who are most susceptible to meningococcal infection. A serotype 2 vaccine can be expected to protect against a significant proportion of disease caused by groups B, C, Y, and W135 (31). Successful application of serotype vaccines will depend upon identification of the serotypes responsible for meningococcal disease in the population to be immunized. The vaccine of choice may well contain multiple serotype proteins as well as group specific capsular polysaccharides since there is evidence for a synergistic effect between protein and polysaccharide antibodies (15).

### Summary

Protein vaccines were prepared from the serotype antigen of group B *Neisseria meningitidis* strain M986. The detergents Triton X-100, Emulphogene BC-720, and deoxycholate were used to remove the toxic lipopolysaccharide (LPS) portion of the serotype antigen. The LPS was most preferentially solubilized by Emulphogene. Guinea pigs were immunized with one or two doses of vaccine given intramuscularly without adjuvants and the antibody response quantitated by an enzyme-linked immunosorbant assay. Immunization with graded doses of vaccine between 25 to 200  $\mu\text{g}$  protein indicated a wide range of effective dosage and that a two-dose immunization schedule was superior to a single immunization. The vaccines elicited peak mean serum antibody levels of approximately 30  $\mu\text{g}/\text{ml}$  with bactericidal titers of 1:1,600–1:6,400. The peak antibody levels occurred 5–6 wk after immunization and persisted above preimmune levels for several months. To evaluate the protective effects of immunization, stainless steel springs were implanted subcutaneously into the guinea pigs. The resulting chambers, in unimmunized animals, could be infected with less than 100 type 2 organisms. A single 25–50  $\mu\text{g}$  dose of vaccine protected 50% of animals from challenge by  $5 \times 10^8$  type 2 meningococci, and as little as 1  $\mu\text{g}$  vaccine significantly reduced the severity of infection. A two-dose immunization schedule was best and provided nearly complete protection for at least 4 mo against type 2 strains of meningococcal groups B, C, and Y.

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