PROTECTION AGAINST GROUP B MENINGOCOCCAL DISEASE

II. Infection and Resulting Immunity in a Guinea Pig Model*

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Exposure to *Neisseria meningitidis* may result in either asymptomatic infection (carriage) or acute disease. Early studies indicated that acute meningococcal disease probably occurs about 2 wk after acquisition of the organism (1). Regardless of whether individuals become carriers or develop acute disease, they develop humoral antibodies to a variety of antigens, including the group-specific polysaccharide (2, 3), the protein serotype antigen (STA),[1] and lipopolysaccharide (LPS) (4). Group-specific polysaccharide antibodies peak approximately 2 wk after onset of acute disease (5, 6), but the nature of the immune response to the STA and LPS antigens resulting from carriage or disease is unknown.

There is no entirely satisfactory animal model simulating meningococcal disease in man, although several have been studied for their ability to predict the effectiveness of vaccines or serum therapy. The mouse-mucin model described by Miller and Castles in 1936 (7) and later standardized by Branham and Pittman (8, 9) was one of the first models for study of immune protection. Study of the immune response resulting from mouse infection is difficult because the effects of mucin on the immune response and clearance are unknown. The rabbit has also been used, but massive doses of organisms injected intracranially were required to cause meningitis-like disease (10). The chick embryo model was used to study passive protection against meningococcal infection (11–14), but active immunization is not possible.

In these studies, the guinea pig implant model of Arko (15) was examined. This model proved useful because (*a*) the animals could be actively immunized, (*b*) useful amounts of serum from individual animals were obtained, (*c*) the induced meningococcal infections were not lethal, thus permitting rechallenge, and (*d*) localization of the infection permitted quantitation of organisms present. As long as no attempt is made to study the pathophysiology in relation to human disease, the guinea pig spring implant model is satisfactory.

The purpose of our investigation was to examine the specificity and duration of the immune response to meningococcal infection and to determine the

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[1] *Abbreviations used in this paper:* BHIA, brain heart infusion agar; ELISA, enzyme-linked immunosorbant assay; LPS, lipopolysaccharide; STA, serotype antigen.

protective effects of the primary infection upon subsequent challenge. These investigations will show that meningococci readily infect the implanted chambers inducing both group- and type-specific protection.

Materials and Methods

Strains and Growth Conditions. Meningococcal group B strains M986, M990, M1080, and M136 have been characterized (16, 17). The group B type 2 strain S946 and group Y type 2 strain S1975 were received from Dr. Harry Feldman, Upstate Medical Center, Syracuse, N. Y. The group C strains 60E and 138I were obtained from the late Dr. Malcolm Artenstein, Walter Reed Army Medical Center, Washington, D. C. For animal challenge, the organisms were grown into log phase (6 h) on brain heart infusion agar (BHIA, Difco Laboratories, Detroit, Mich.) at 36°C in candle jars. The organisms were diluted in Hanks' balanced salt solution to 80% transmission at 540 nm in a Coleman Jr. spectrophotometer (3×10^8/ml). After challenge, fluid aspirated from the guinea pig chambers was diluted 10^{-1} and 10^{-3} in Hanks' balanced salt solution and cultured onto BHIA. For some experiments, blood and chamber fluid were cultured onto serum agar plates containing 6% horse anti-group B serum. On these plates, group B meningococci were identified by a precipitin halo surrounding the colonies after 24–48 h growth at 36°C.

Guinea Pig Model. Stainless steel springs were implanted into 400–600 g Hartley (Rockefeller University, New York) and NIH-Hartley guinea pigs as described by Arko (15). The 1 cm × 2.5-cm spring chambers were prepared from 22 B&S gauge surgical steel (Perkin-Elmer Corp., Maywood, Ill.) and implanted subcutaneously on one or both sides of the animals through a midline incision on the back. Log phase meningococci were injected either intravenously or directly into the chambers 10–12 days after implantation. The chambers were usable up to 3 wk after the initial challenge, after which they failed to allow meningococcal growth. Chambers were always sampled 2 days after challenge and various days thereafter by removing 0.1 ml fluid with a 1 ml disposable syringe. Animals were considered infected if on day 2 they had more than 100 organisms/milliliter in their subcutaneous chambers.

Most animals were bled from the heart for antibody determinations, and some from an ear vein for blood culture.

Serological Methods. The enzyme-linked immunosorbant assay (ELISA) was used to measure serotype antibody by the technique of Engvall and Perlmann (18) with slight modification (4). Indirect hemagglutination was performed as described (19), with purified meningococcal polysaccharides.

Results

Implantation of stainless steel springs into guinea pigs provided an artificial subcutaneous tissue cavity in apparent free association with the animals' humoral and cellular defense mechanisms. Preliminary experiments were done to characterize the infectivity and dose required to induce a 100% infection rate (ID_{100}). Results of three such infectivity assays are shown in Table I. The organisms used for infectivity experiments were not passaged through guinea pigs to increase their virulence. A challenge dose of approximately 10^5 organisms for the following experiments was chosen to ensure 100% infection in nonimmune animals.

Duration of infection was determined for several different serotype strains (Table II). Most strains examined persisted for several days at approximately 5×10^6 organisms/ml of chamber fluid, even though within 24 h of infection, large numbers of polymorphonuclear leukocytes were present in Gram-stained smears of the fluid. The number of organisms in the chambers remained relatively constant for 4–6 days, followed by elimination of infection by approximately day 12. Three group Y strains (S-1975, S-1255, and S-1161) were

TABLE I
Infectivity of Different Group B Strains for Spring Implants in Guinea Pigs

Animal set	Strain	Serotype	No. organisms injected	Organisms/ml day 2*	Animals infected/ challenged
15	M1080	1	2×10^4	2×10^6	3/3
16			2×10^3	5×10^4	2/3
17			2×10^2	1×10^4	2/3
18			2×10^1	5×10^4	2/3
C	S-946	2	3×10^5	1×10^7	3/3
4			4×10^4	2×10^7	4/4
5			4×10^3	3×10^7	4/4
6			4×10^2	9×10^6	4/4
19	M-990	6	4×10^5	1×10^7	3/3
20			4×10^4	2×10^7	3/3
21			4×10^3	2×10^7	3/3
22			4×10^2	2×10^7	2/3

* Average number of organisms/milliliter in chamber was determined 2 days postinfection and calculated based upon those infected.

TABLE II
Duration of Infection after Challenge by Different Meningococcal Strains

Animal set	Strain	Serogroup, serotype	Challenge dose	No. infected/challenged on days (Average organisms/milliliter)						
				2	4	6	8	10	12	14
15	M1080	B, 1	2×10^4	3/3 (2×10^6)	–*	3/3 (2×10^4)	–	–	0/3	–
23	S-946	B, 2	5×10^5	4/4 (7×10^7)	–	4/4 (1×10^7)	–	–	2/4 (4×10^2)	–
12	138I	C, 2	2×10^6	4/4 (4×10^7)	–	–	4/4 (2×10^6)	–	1/4 (6×10^3)	–
30	S-1975	Y, 2	4×10^5	4/4 (5×10^3)	3/4 (2×10^4)	3/4 (1×10^4)	–	1/4 (3×10^4)	–	0/4
20	M990	B, 6	4×10^4	3/3 (2×10^7)	–	2/3 (7×10^2)	–	–	0/3	–
11	M136	B, 11	2×10^6	3/3 (8×10^6)	–	–	3/3 (4×10^5)	–	0/3	–

* Not done.

examined and all persisted as long as other strains, but never at concentrations greater than 10^4 organisms/milliliter.

For protection studies, we initially considered rechallenge of animals simultaneously with two different meningococcal strains injected into two widely spaced subcutaneous chambers as reported for gonococci (20, 21). The feasibility of this technique was tested by injecting 10^5 organisms of strain S-946 (group B, type 2) into one chamber 14 days after implantation of 2 chambers. Contrary to our expectations, both chambers were equally infected (10^7 organisms/ml) 2 days after challenge. It was clear from this experiment that simultaneous challenge with different strains was not feasible. To determine whether hematogenous spread could account for these results, sets of animals, each with two implants, were challenged with S-946 either intravenously, or directly into their right chambers (Table III). 1 day after challenge, 10 meningococci/ milliliter were cultured from the blood of two of eight animals challenged

TABLE III

Role of the Spring Implant as a Nidus for Initiation of Meningococcal Infection by Group B Type 2 Strain S-946

Animal sets	Challenge		Days post challenge	Infection of chambers				Blood infection
				Right		Left		
	Dose	Route		Infected/challenged	Average organism/ml	Infected/challenged	Average organism/ml	
35, 36	2×10^8	i.v.	1	6/8	3×10^5	6/8	2×10^5	2/8
			2	7/8	9×10^6	8/8	1×10^7	2/8
			6	8/8	4×10^6	7/8	3×10^6	–
37, 38	4×10^5	Chamber (right)	1	8/8	6×10^4	6/8	1×10^4	0/8
			2	6/8	3×10^6	5/8	1×10^6	0/8
			6	6/8	5×10^6	6/8	2×10^6	–

intravenously, and by day 2 all had infected chambers. Of eight animals challenged directly in the right chamber, six became infected in the left chamber. Thus, the chamber implant probably serves as a nidus in which an infection can be established by hematogenous spread. Direct subcutaneous extension between chambers is an alternative or second mode of spread. Support for this possibility is our finding that greatly reduced transfer between chambers occurred when the right chamber was not challenged until 4 wk after implantation (chambers do not fibrose as rapidly in unchallenged animals).

Animals were examined for their resistance to homologous reinfection with a challenge dose of approximately 100 times the MID_{100}, given 13–90 days after clearance of the primary infection (Table IV). All animals were resistant to homologous rechallenge and they remained so for at least 3 mo.

The accelerated clearance of meningococci 8–10 days after infection suggested immune clearance. To determine the relationship between clearance of infection and appearance of humoral antibody, guinea pigs were prebled and then infected with strain S-946. Cultures and sera were obtained at intervals until the infection was eliminated (Fig. 1). Coincident with elimination of the infection was a rise in serotype antibody. Although antibody levels on day 14 were low, they continued to rise after the infection cleared (Fig. 2). Antibody to the group B polysaccharide as measured by indirect hemagglutination was also present by day 14.

The antibody response to the type 1 and type 6 STAs was determined by ELISA in two sets of animals, one infected with strain M1080 (B, type 1), and the other with M990 (B, type 6). The results are shown in Fig. 2. Although the infections were eliminated within 2 wk in both groups of animals, their antibody levels did not peak until 4 wk later, and by 4 mo the antibody had still not returned to preinfection levels. Cross-reactivity in the antibody response as measured by ELISA was observed previously in rabbit sera (22).

Since disease-producing meningococci of several different groups (B, C, Y, and W135) may possess serotype 2 antigen (23), it was important to know whether the observed protection in the guinea pigs would prove to be group and/or type-specific. Sets of animals were initially infected with type 2 strains of groups B, C, and Y, and an additional set with a group B type 11 strain

TABLE IV

Resistance of Animals to Rechallenge with the Homologous Meningococcal Strain

Animal set	Challenge strain	Serogroup, serotype	Challenge dose	Days after primary infection*	Animals infected/challenged
6	M986	B, 2	3×10^5	45	0/3
6	S-946	B, 2	4×10^6	26	0/3
23	S-946	B, 2	4×10^5	90	0/3
12	138I	C, 2	1×10^7	13	0/4
30	S-1975	Y, 2	1×10^8	17	0/4
11	M136	B, 11	3×10^6	13	0/3

* Number of days after clearance of primary infection at time of rechallenge.

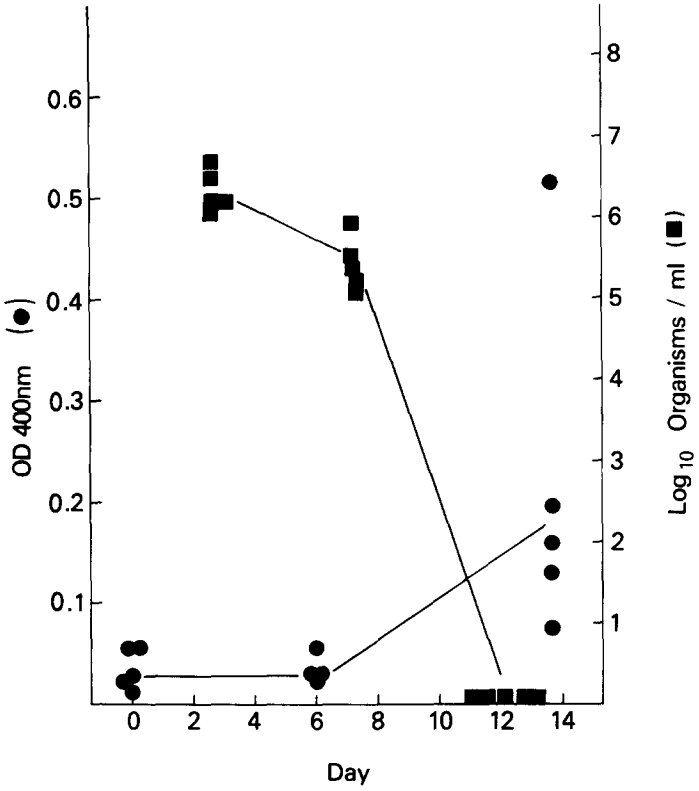

FIG. 1. Correlation of serotype antibody formation as measured by ELISA with elimination of infection by strain S-946 (B, 2).

(Table V). The challenge dose for all strains was approximately 6×10^5 log phase organisms, except for group Y, which was 10^8 organisms, and was given 3 wk after clearance of the primary infection. Animals initially infected with type 2 strains of groups B, C, or Y resisted rechallenge with type 2 strains, regardless of serogroup. There was also group-specific protection since animals infected with two different group B strains resisted rechallenge with one or two heterologous group B strains but did not resist reinfection by a group C strain

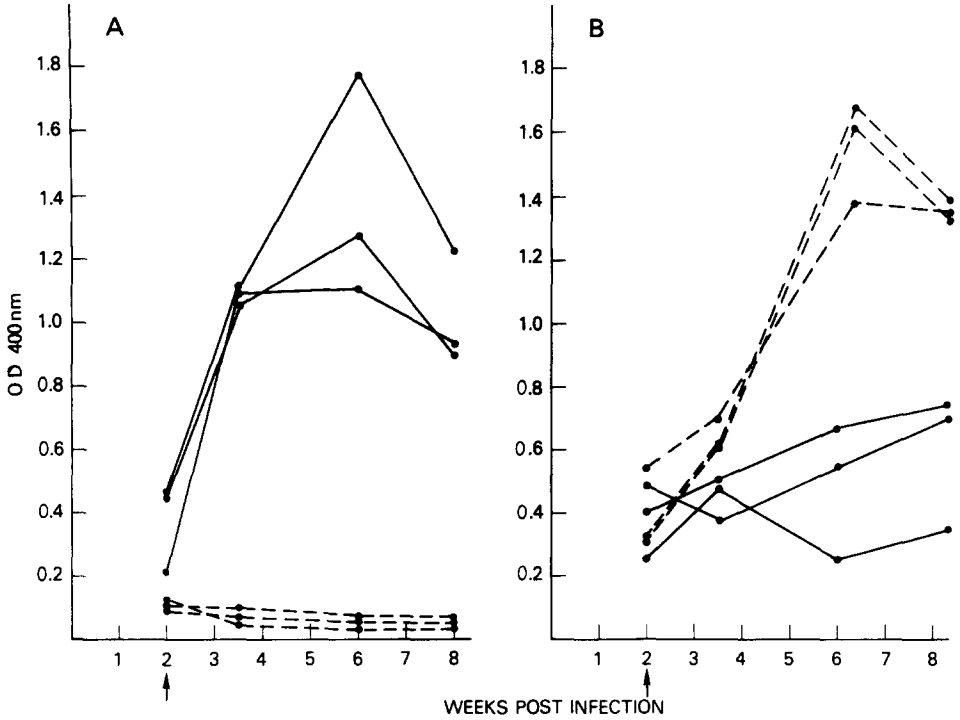

FIG. 2. Serotype antibody response as a result of infection with group B meningococcal strains. (A) Guinea pigs infected at zero time with strain M990 (B, 6). Homologous antibody response to type 6 STA is solid line and cross reacting response to type 1 STA is broken line. (B) Guinea pigs infected with strain M1080 (B, 1). Homologous antibody response to type 1 STA is broken line and cross reacting response to type 6 STA is solid line. The arrow on both A and B shows when the infections were eliminated.

of heterologous serotype. Thus, both group- and type-specific protection was observed.

There was a marked difference in the course of infection observed in resistant and nonresistant animals. Animals that became infected upon rechallenge with group C strain 60E had approximately 10^7 organisms/milliliter at 2 days and the infections persisted 10–12 days. In contrast, those animals who were partially immune but became infected had only 10^2–10^3 organisms/milliliter at day 2, the infection never persisting beyond day 4.

We examined sera from animals infected with group B or group C strains to determine whether the observed group-specific protection was due to antibodies against the capsular polysaccharide (Table VI). There was a significant increase in anti-capsular antibody, the peak polysaccharide antibody response occurring much earlier than the response against the protein antigens (see Fig. 2).

Discussion

In our previous study on passive protection against group B meningococcal infection in the chick embryo (13), both group-specific and type-specific protection were observed. Moreover, there was a synergistic effect between group-

TABLE V

Specificity of Protection after Infection with Different Meningococcal Strains

Animal Set	Primary infection		Challenge with strain (group, type)*					
	Strain (group, type)	No. infected	M986 (B, 2)	M990 (B, 6)	M136 (B, 11)	138I (C, 2)	60E (C, NT)	S-1975 (Y, 2)
b	S-946 (B, 2)	3/3‡	0/3‡	–	–	–	–	–
c	S-946	3/3	–	0/3	–	–	–	–
a	S-946	3/3	–	–	0/3	–	–	–
4	S-946	4/4	–	–	–	1/4	–	–
6	S-946	3/3	–	–	–	–	3/3	–
11	M136 (B, 11)	3/3	0/3	–	0/3	–	3/3	–
12	138I (C, 2)	4/4	0/3	–	–	0/4	3/3	–
30	S-1975 (Y, 2)	4/4	0/4	–	–	1/4	–	0/4

* All animals were challenged with approximately 5×10^5 log phase organisms into a chamber.
‡ Number infected/challenged determined on day 2.

TABLE VI

Antibody Response to the Capsular Polysaccharide as a Result of Infection in Guinea Pigs

Animal set	No. animals	Infecting strain	Group, type	Serum on week	Mean titer against*	
					B	C
1	3	–	–	–	<2	<2
15	3	M1080	B, 1	2	21 ± 9	5 ± 3
				6	9 ± 7	2 ± 2
23	3	S-496	B, 2	2	37 ± 24	3 ± 1
				6	15 ± 15	1 ± 1
12	4	138I	C, 2	1	1 ± 2	13 ± 5
				2	<2	11 ± 5

* Titer determined by indirect hemagglutination of sheep erythrocytes coated with purified polysaccharide.

and type-specific antibody. However, this model has definite disadvantages: the chick embryo cannot be actively immunized, and it lacks an active complement system (24) which is critical in immune defense against meningococcal disease (25, 26). We, therefore, looked for an animal model that could be actively immunized by either prior infection or vaccination.

The guinea pig chamber model has been used to study *Neisseria gonorrhoeae* infection (15, 20, 27, 28), but *N. meningitidis* infection of these chambers was not examined. Various materials have been used to establish artificial chambers for Neisserial infection of guinea pigs (15, 20, 27). We chose the stainless steel spring for implantation because it allows the most freedom of interaction of host cellular and humoral immune mechanisms with the *Neisseria*, whereas plastic chambers may become highly encapsulated and allow the establishment of persistent infections (27, 28).

For most meningococcal strains we could achieve an ID_{100} with 10–1,000 times fewer organisms than reported for fresh unpassaged gonococcal isolates

(27, 29). As we had observed in our chick embryo emperiments (13), group B serotype 2 strains infected at a lower challenge dose and persisted in greater numbers than many other group B strains. Three group Y, type 2 strains required much higher challenge doses and the number of organisms per milliliter in the chamber at days 2–4 were only 10^4 vs. 10^7 for B, 2 strains, suggesting a lower virulence of group Y strains. Calver et al. (30) also found that a group Y strain had a much higher LD_{50} for iron-treated mice than A, B, or C strains. Therefore, infection in these animal models appears to correlate to some extent with the epidemiological data in humans because there is a strong association of serotype 2 with meningococcal disease (31–33) and high carrier rates are often observed for group Y organisms in absence of group Y disease (34).

Duration of infection after inoculation of stainless steel spring implants, placed in animals 10–14 days previously, was comparable for meningococcal and gonococcal strains (35). Guinea pigs infected with various meningococcal strains usually cleared their infections in 10–12 days. Clearance of infection correlated with appearance of serotype and serogroup antibody. In unpublished observations, we found that serotype antibody elicited by guinea pig infection was bactericidal in vitro.

Resistance to reinfection was determined with a single challenge dose, estimated to be approximately 100 times the uniformly infectious dose. Other investigators have reported using graded challenge doses given over a period of several days (20, 35). This latter method is less satisfactory because the preinfecting challenge doses may potentiate the apparent immunity to infection in already immunologically primed animals.

Resistance of the guinea pigs to rechallenge after clearance of their primary infection was both group- and type-specific. The levels of serotype antibody induced as a result of infection were considerably lower than those after immunization with serotype vaccines,[2] yet both protected against meningococcal challenge.

Summary

A guinea pig subcutaneous chamber model was used to evaluate the specificity of the immune response resulting from *Neisseria meningitidis* infection. Small numbers of meningococci easily infected the chambers. The infections persisted for 6–8 days with relatively high levels of organisms (10^5–10^6/milliliter) in the chambers, and were then rapidly eliminated and no organisms could be cultured beyond day 14. Clearance of infection correlated with appearance of circulating antibody. Antibody against both the protein serotype antigen and the capsular polysaccharide were induced as a result of meningococcal infection. The group-specific polysaccharide response peaked 2–3 wk after the animals were inoculated, while the type-specific protein response peaked at 5–6 wk. The animals were quite resistant to reinfection with either the homologous serogroup or serotype.

[2] C. E. Frasch, and J. D. Robbins. 1978. Protection against group B meningococcal disease. III. Immunogenicity of serotype 2 vaccines and specificity of protection in a guinea pig model. *J. Exp. Med.* **147**: 629.

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