HUMAN IMMUNITY TO THE MENINGOCOCCUS

I. The Role of Humoral Antibodies

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Meningococcemia and meningococcal meningitis in man appear to be infrequent complications of the host-parasite relationship. Ordinarily, exposure to a strain of *Neisseria meningitidis* results in carriage of the organism in the nasopharynx for weeks or months (1–4), producing either a mild pharyngitis or no symptoms at all (5, 6). Even during an epidemic, the great majority of individuals exposed to the epidemic strain of meningococcus become asymptomatic carriers rather than clinical cases with systemic disease (7). Viewed in this light, the occurrence of meningococcal disease is related more to the unique susceptibility of the individual host than to the innate virulence of the infecting organism.

Several epidemiological findings suggest that individuals who are susceptible to systemic meningococcal disease lack humoral antibodies to meningococci. First, except for outbreaks among military recruits (8, 9) and other closed populations (10), meningococcal meningitis is a disease of infancy and early childhood (11, 12). This is true both in epidemic and interepidemic periods. Susceptibility to many so-called "diseases of childhood," bacterial and viral, has been shown to be associated with deficient levels of circulating antibodies to the infecting microbe (13). Second, the cyclical occurrence of epidemic meningococcal disease in the United States at approximately 10 yr intervals (14) suggests that the disease depends for its expression on the presence of an immunologically virgin population. Third, meningococcemia seems to confer permanent immunity to future attacks of systemic meningococcal disease, although this is difficult to test statistically (10, 15). Convalescent sera from such cases have elevated levels of precipitating, agglutinating, complement-fixing, opsonizing, bactericidal, and mouse-protective antibodies to the infecting meningococcus (16–18).

The absence of a suitable animal model for the experimental study of meningococcemia and meningococcal meningitis has impeded the evaluation of the host-parasite relationship. Antibodies to meningococci have been found to be present normally in blood from mouse, rat, guinea pig, rabbit, rhesus monkey, chimpanzee, horse, and ox (19).[1] It is possible that these antibodies are responsi-

[1] Goldschneider, I. Unpublished observations.

ble, at least in part, for the insensitivity of such animals to meningococcal disease. Similarly, antimeningococcal antibodies have been found in normal human blood (19–22). These observations, together with the epidemiological findings presented above, suggest that humoral antibodies may be important in preventing systemic meningococcal disease in man.

Such an idea is not new. Kolmer et al. (23) showed that many normal human sera contain opsonic antibodies to meningococci isolated from cases of meningitis, and postulated that such antibodies might be protective. Heist et al. (24) found that blood from more than 95% of normal men was bactericidal to meningococcal strains obtained from asymptomatic carriers. They postulated that most cases of meningitis occur among those few individuals who lack bactericidal activity. Dr. Heist, whose own blood was nonreactive against meningococci, died with meningococcal meningitis. While these early experiments were suggestive of a protective role of circulating antibody against meningococcal disease, definitive evidence was lacking. The presentation of such evidence is the subject of this paper.

Methods

Bacteriological Techniques.—

Bacteria: Strains of *Neisseria meningitidis* were obtained from the collection of the Department of Bacteriology, Walter Reed Army Institute of Research, and from surveys conducted at several military installations, as indicated in the text. Specimens from blood and cerebrospinal fluid were plated on "chocolate" agar (Mueller-Hinton base [25], Difco Laboratories, Detroit, Mich.), and those from nasopharynx on "chocolate" agar containing 6 μg lincomycin hydrochloride monohydrate (Upjohn Co., Kalamazoo, Mich.) and 25 units polymyxin B sulfate (Charles Pfizer & Co., New York) per milliliter (26). The plates were incubated for 16 to 24 hr at 37°C in the presence of moisture and CO_2 (candle jar). Bacterial isolates were subcultured once on chocolate agar, without antibiotics, and identified as meningococci by serogrouping and fermentation of carbohydrates. The strains were preserved by lyophilization, and by freezing in a preserving solution at −70°C. The preserving medium (27) consisted of 5% (w/v) bovine serum albumin (Calbiochem, Los Angeles, Calif.) and 5% (w/v) monosodium glutamate (Sigma Chemical Co., St. Louis, Mo.) in distilled water. Renewal of the working (frozen) stock was made from the lyophile collection. Thus, all meningococcal strains used in the present experiments were within two passages of original isolation.

Serogrouping: The slide agglutination technique (28) was employed for routine serogrouping of meningococci. Commercially obtained typing sera (Difco) were used to identify group A, B, and C organisms; and rabbit antisera prepared in this laboratory against prototype organisms were used to type group Boshard (Bo), 135, and 29-Eur (29), and group X and Z (30). Serogroups Bo and Y (30) were considered to be identical (29). Meningococci which agglutinated spontaneously in saline, which were agglutinated by antisera to several serogroups, or which were not agglutinated by any serum in the panel were called "nontypable" (NT).

Carbohydrate fermentation: The bacterial isolates were tested for carbohydrate fermentation by a replicate plating method using a modified Lidwell phage applicator (31). Each strain was tested against dextrose, maltose, sucrose, and lactose. Only bacteria which had typical colonial morphology and fermented dextrose and maltose, or dextrose alone in the case of some sulfonamide-resistant organisms (32), were classified as *N. meningitidis.*

Preparation of bacterial suspensions: Bacteria were cultured on "chocolate" agar (Mueller-Hinton base, Difco lot No. 496202) for 16 hr at 37°C in the presence of moisture and CO_2 (candle jar). The organisms were subcultured for 5 hr on fresh chocolate agar and suspended in Dulbecco's (33) phosphate-buffered saline (PBS) pH 7.2 (Grand Island Biological Co. Grand Island, New York). The concentration was adjusted to an optical density of 0.20 at

TABLE I

Sources of Children's Sera

Group No.	Location	Donors		
		Age range	Clinical history	No. sera
1	Beth Israel Hosp., Boston (Dr. Ronald Gold)	Newborn	Normal	12
2	Univ. of Miami, Florida (Dr. Bernard Fogel)	Newborn—6 months	Normal	45
3	Georgetown Univ., Washington, D. C. (Dr. Joseph Bellanti)	6 months–2 yr	Recurrent upper respiratory infections	16
4	Walter Reed Gen. Hosp., Washington, D. C. (Dr. Wm. Stewart)	6 months–12 yr	Normal	45
5	Hosp. for Sick Children, Washington, D. C. (Dr. Joseph Bellanti)	6 months–12 yr	Chronic noninfectious illnesses	48
6	Junior Village, Washington, D. C. (Dr. Albert Kapikian)	6 months–12 yr	Normal	79
7	Div. Biol. Standards, N. I. H., Bethesda, Md. (Dr. Paul Parkman)	7 months–12 yr	Normal	37
Total....				282

650 mμ (16 × 125 mm Pyrex screw cap tube, Meteor Glass Co., Vineland, N. J.) in a spectrophotometer (Coleman Jr., model 6A). This corresponded to a colony count of approximately 10^9 bacteria/ml. Appropriate dilutions were made in PBS. It was found that the total particle count, as measured in a hemocytometer, approximated (±10%) the viability count (colony count).

Sera.—Human blood was collected by venipuncture, allowed to clot for 60 min at room temperature, and refrigerated at 4°C for 3 hr. After centrifugation (1500 *g* for 10 min) the sera were placed in sterile 5 or 9 ml screw cap vials and stored at −70°C. Sera were not refrozen more than three times. $C'H_{50}$ hemolytic units (34) were determined on all sera defrosted more than once.

Children: Table I shows the sources of sera from children. None of the children were receiving antibiotics, gamma globulin, or immunosuppressant drugs.

Adults: Serum was obtained from army recruits (ages 19–26 yr) during their 1st wk of basic training at Fort Dix, N. J., 1966–1968. Blood was drawn prior to the routine post-induction immunizations.

Gamma Globulin.—Lyophilized, pooled human gamma globulin (Cohn fraction II, Lot No. 2191) was obtained from E. R. Squibb, Inc., New Brunswick, N. J. The gamma globulin was dissolved in PBS at a concentration of 10% (w/v) and centrifuged at 105,000 *g* (Spinco ultracentrifuge, model L) for 5 hr. The upper two-thirds of the supernate was retained, stored at 4°C, and used within 24 hr of preparation.

Complement.—The source of complement was normal human serum (P. M.) which lacked bactericidal activity to the strains of *N. meningitidis* used in this study. The serum was divided into aliquots, stored at −70°C, defrosted immediately prior to use and not refrozen. The donor was cultured repeatedly to ascertain that he did not become a carrier of meningococci during the study.

In all instances in which exogenous complement was *not* added to the bactericidal system $C'H_{50}$ units/ml were determined by the method of Hook and Muschel (34). Only sera having 140 or more $C'H_{50}$ units/ml were considered in tabulating results. Amboceptor-coated sheep red blood cells were kindly provided by Mr. Earl Fife, Department of Serology, Walter Reed Army Institute of Research.

Antibody Assays.—

1. Serum bactericidal reaction: The serum bactericidal test was performed in a Microtiter system (35) using disposable U-well trays (Linbro Chemical Co., New Haven, Conn.), and 25 and 50 μl droppers and 25 and 50 μl diluters (Cooke Engineering Co., Alexandria, Va.). Plastic materials were sterilized by exposure to ethylene oxide (Anprolene, C. R. Bard, Inc.) for 16 hr. The diluters were flamed and allowed to cool prior to use.

The reaction mixture had a total volume of 0.2 ml and consisted of one part diluted serum (or gamma globulin), one part PBS, one part complement (or PBS), and one part bacterial suspension (approximately 10^4 bacteria/ml), added in that order. Dulbecco's phosphate-buffered saline (10^{-3} M Ca^{++}, 5×10^{-4} M Mg^{++}) was the diluent. Complement and heat-inactivated serum controls (56°C for 30 min) were included in each experiment.

The tray was sealed with sterile (UV irradiation) transparent tape (Cooke Engineering Co.), forcefully inverted to mix the reactants, and incubated at 37°C for 30 min. After removing the seal, the Microtiter tray was placed on edge and supported at an angle of approximately 75° to horizontal. Fluid from each well was allowed to flow into a glass capillary (Fisher Scientific Co., Pittsburgh, Pa., No. 12-141), and a drop was deposited on Mueller-Hinton agar which had been partially dehydrated by storage at room temperature for 4 to 8 days. Rapid absorption of fluid by the agar terminated any residual bactericidal activity and also prevented dispersion of dividing bacteria. The capillaries delivered approximately 0.02 ml per drop. In the absence of bactericidal activity such a drop contained approximately 50 colony-forming units. The inoculated plates were incubated at 37°C for 18 to 24 hr and colony counts performed.

a. Interpretation of bactericidal test: Replicate colony counts in several hundred experiments showed the error in the system to be ±20%. For this reason, only colony counts which were less than 50% of control levels were considered indicative of bactericidal activity. The 50% level of killing was also chosen to maximize the sensitivity of the bactericidal assay.

The 30 min incubation period for the bactericidal reaction was selected for three reasons. First, sequential colony counts showed that the serum bactericidal activity against meningococci was completed by 30 min (usually by 15 min). Second, meningococci placed in lag phase by suspending in PBS at room temperature did not divide in the complement or heated-serum controls during the 30 min incubation period, but did so after 60 min. Third, viability counts

of meningococci in PBS (or high serum dilutions) remained constant for 60 min, but spontaneously decreased thereafter.

Agglutination of bacteria did not present a problem in the interpretation of colony counts after exposure of meningococci to serum. In low titer sera (bactericidal activity in dilutions less than 1:32) there was no significant agglutination. Such sera, when decomplemented by heating at 56°C for 30 min, did not produce any depression in colony counts compared to complement or PBS controls. However, antibodies were apparently unaffected by the heat inactivation as evidenced by restoration of bactericidal activity after addition of complement, and by continued binding of specific IgG, IgM, and IgA antibodies to meningococci as determined by indirect immunofluorescence (*vide infra*). Agglutination *did* occur in some high titer sera, but was of no practical consequence in the interpretation of bactericidal activity. With few exceptions, there was an abrupt transition between serum dilutions producing 100% killing and those showing 0% killing.

Consistent results in the serum bactericidal system were obtained if there was strict adherence to the procedures outlined. However, deviation in age of the bacterial culture (less than 4 hr or more than 6 hr), use of a different lot number of Mueller-Hinton agar, or repeated passage of the meningococcal strain in vitro affected the reproducibility of the system, (i.e., increased or decreased sensitivity of the meningococcal strain to the lethal action of antibody and complement). For this reason, comparable experiments were done at one time, or if this was not possible, representative specimens were included as controls in subsequent experiments.

2. Immunofluorescence: Indirect immunofluorescence was done using standard techniques (36). The antigens were 5 hr cultures of meningococci, washed once with distilled water, suspended in distilled water at a concentration of 2×10^8 bacteria/ml, and dried onto slides at 37°C. Human sera were used in a standard dilution of 1:2 or titrated by serial 2-fold dilutions. Fluorescein-conjugated rabbit antisera to heavy chains of human IgG, IgM, or IgA globulins were used in a 1:20 dilution (Behringwerke, Marburg-Lahn, Germany; distributed by Hoechst Pharmaceutical Co., Kansas City Mo.). Phosphate-buffered saline, pH 7.2 was the diluent throughout.

The antigen was incubated at room temperature with human serum for 20 min, washed in three changes of PBS (5 min each), reincubated for 20 min in fluorescein-conjugated rabbit antiserum, rewashed, and mounted in buffered glycerin (Difco). Specimens were examined in a Zeiss Standard Universal microscope (Carl Zeiss, Inc., Oberkochen, West Germany), equipped with a 100 × apochromatic oil immersion objective with iris diaphragm, 8 × eyepiece and 1.25 × Optovar magnifying attachment. The ultraviolet light source was a 200 w mercury vapor arc lamp. A BG 12 excitation filter and a combination of barrier filters Nos. 53 and 44 were used.

The sensitivities of the bactericidal and immunofluorescence (anti-IgG) assays for the detection of antimeningococcal antibodies in human sera were similar (± one 2-fold serum dilution).

RESULTS

Age-Related Immunity—Fig. 1 shows the relationship between serum bactericidal activity against *N. meningitidis* and the incidence of systemic meningococcal disease in the general population, newborn to 26 yr of age. Three strains of meningococci, A1 (serogroup A), B11 (serogroup B) and C11 (serogroup C) from cases of meningitis were chosen as prototypes. These are the serogroups responsible for over 90% of cases of meningococcal meningitis (37). Age-specific morbidity rates for meningococcal infections in the United States in 1965 and

1966 were obtained from the National Communicable Disease Center, Atlanta, Ga.[2] Attack rates were calculated using estimated population bases for the various age groups (38). In addition, a survey of the age distribution of 72 cases of meningococcal meningitis admitted to Los Angeles Children's Hospital (1944–

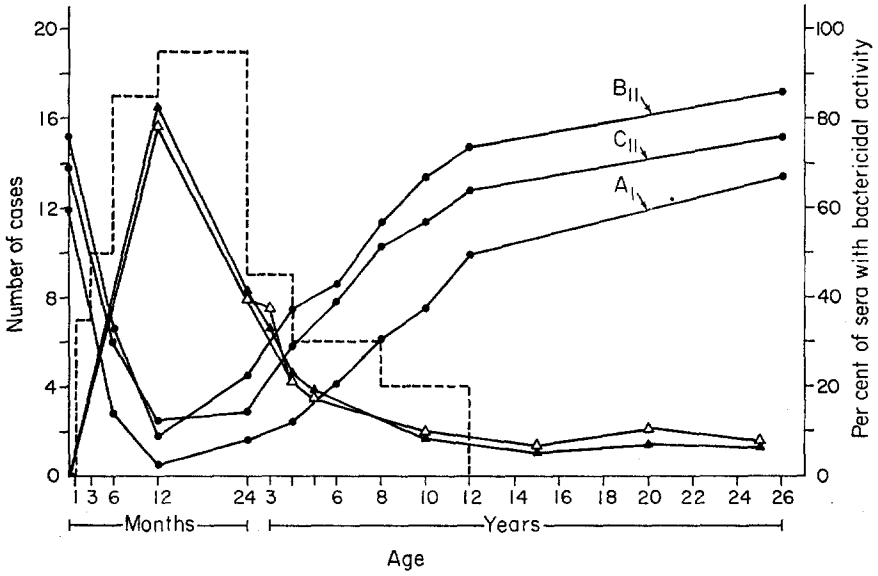

FIG. 1. Age-related incidence of meningococcal disease in the United States and prevalence of serum bactericidal activity against three pathogenic strains of *N. meningitidis*. ▲——▲, number of cases/100,000 age-specific population, 1965; △——△, number of cases/100,000 age-specific population, 1966; ———, age distribution of 72 cases admitted to Los Angeles Children's Hospital 1944–1953 (adapted from Smith [39]); ●——●, per cent of sera in each age group having a bactericidal titer of 1:4 or greater against meningococcal strains A1, B11, and C11. Sera from 282 children (at least 20 in each age group) and 567 army recruits (ages 19 to 26 yr) were tested in the presence of exogenous complement. Each point in the figure represents the incidence of disease or prevalence of bactericidal activity among subjects in the age range encompassed by it and the previous point (e.g., 2.5% of children 6–12 months of age had serum bactericidal activity against meningococcal strain A1).

1953) (39) was included to provide more detailed data on the incidence of meningococcal disease during the first 2 yr of life. Inasmuch as two epidemics of meningococcal meningitis occurred between 1944 and 1953 (14), it is reasonable to assume that many of the cases among these children were caused by serogroup A (40). Cases occurring in 1965 and 1966 were due in the main to meningococcal serogroups B and C (40).

[2] Courtesy of Ida Sherman, Acting Chief, Statistics Section, Epidemiology Program.

The data in Fig. 1 show that meningococcal disease is uncommon in the 1st month of life, but that significant numbers of cases begin to appear during the 2nd and 3rd months. The peak incidence of meningococcal meningitis occurs between 6 months and 2 yr of age, after which there is progressive decrease in attack rate. A second, smaller peak occurs in the young adult population. This is due almost entirely to cases of meningococcal disease among military recruits (42–44). After age 25, the attack rate per year fluctuates between 0.3 and 0.5/100,000 population.

The per cent of individuals having bactericidal activity in their serum against the prototype strains of *N. meningitidis* is inversely proportional to the incidence of meningococcal meningitis during the first 12 yrs of life. Results in Fig. 1 show that sera from more than 50% of newborn infants have bactericidal titers of at least 1:4 against one or more of the meningococcal test strains. The prevalence of bactericidal activity decreases rapidly after birth and reaches its lowest level between 6 months and 24 months of age. From 24 months to 12 yrs of age, there is an essentially linear increase in per cent of sera having bactericidal activity. By ages 19–26 yr, serum from approximately 67% of incoming military recruits has bactericidal activity against the serogroup A meningococcus, 86% against serogroup B, and 76% against serogroup C. These figures are only slightly higher than those for serum from newborn infants.

Serum Bactericidal Activity and Susceptibility to Systemic Meningococcal Disease.—It seems reasonable to hypothesize, on the basis of data presented in Fig. 1, that susceptibility to meningococcal meningitis is related to a deficiency of humoral antibodies as measured by the serum bactericidal test (45, 46). In order to test this hypothesis, a prospective study was started among incoming recruits at the U.S. Army base, Fort Dix, N. J. Recruits were bled during the 1st wk of basic training and the sera stored at −70°C in anticipation of the occurrence of meningococcemia or meningococcal meningitis among some of the men during the 8 wk training period. This serum is referred to as *base line serum*, as opposed to acute phase serum collected on the day of onset of clinical disease (often after parenteral administration of antibiotics). In all, 14,744 recruits were bled between 1 December 1967 and 31 March 1968, among which 60 cases of systemic meningococcal disease occurred. In every instance in which the organism was isolated, it belonged to serogroup C. At the peak of the meningitis outbreak in February, the attack rate reached 0.5 per 100 recruits per 4 wk training—a rate approximately 300 times greater than that current in the civilian population of the United States (47).

Base line serum from each case was tested for bactericidal activity against the strain of meningococcus isolated from his blood or cerebrospinal fluid (homologous strain). 10 control base line sera, selected at random from men in the same training platoon as the case, were also tested against the meningococcal isolate. There were 46 instances in the prospective study in which both base line serum

and the infecting meningococcal strain were available for study. In addition, base line sera from five group C cases of meningitis which occurred at Fort Dix, winter 1966 and spring 1967, and sera from three cases occurring in April 1968 were included, along with appropriate control sera. Results of this study are summarized in Table II.

It is clear from the data that base line sera from cases of systemic meningococcal disease lack bactericidal activity to the homologous disease-producing strain of meningococcus. Only 5.6% of base line sera from cases had bactericidal

TABLE II

Meningococcocidal Activity of Base Line Sera from Prospective Cases of Meningococcal Disease*

Meningococci	Bactericidal titer 1:4 or greater		Statistical significance
	Base line sera from cases	Base line sera from controls	
	No. positive/total (%)		
Homologous strains‡	3/54 (5.6)	444/540 (82.2)	$P < 0.001$§
Other pathogenic strains			
A1 (serogroup A)	4/23 (17.4)	166/230 (72.2)	$P < 0.001$‖
B11 (serogroup B)	3/23 (13.0)	179/230 (77.8)	$P < 0.001$‖
C11 (serogroup C)	2/23 (8.7)	154/230 (67.0)	$P < 0.001$‖
Strain from carrier (G-2-81) (Serogroup Y)	21/23 (91.3)	221/230 (96.1)	$P > 0.750$‖

* Obtained from recruits in 1st wk of basic training at Fort Dix, N. J., 1967–1968.

‡ Isolated from patients whose base line sera were tested. Base line serum from each case of meningococcal disease was tested against his own meningococcal strain. Base line sera from 10 controls were tested against each strain (total 540 control sera).

§ Calculated using Fisher's exact test for 2 × 2 tables (63).

‖ Calculated by the method of χ^2 (63).

activity in dilutions of 1:4 compared to 82.2% of control sera. Further, results in Table II indicate that base line sera from cases are relatively deficient in their ability to kill disease-producing strains of meningococci in general. Thus, the proportion of base line sera from cases able to kill prototype strains A1, B11, and C11 was significantly lower than that of controls. The mean titer of control sera having bactericidal activity against meningococcal strain A1 was 1:16 (range 1:4 to 1:128). However, when tested against meningococcal strain G-2-81 (serogroup Y) isolated from the nasopharynx of an asymptomatic carrier, base line sera from cases killed as frequently as did control sera.

Immunological Deficit in Sera from Susceptible Host Population.—There are several possible explanations for the inability of base line sera from cases of meningococcal meningitis to kill their own and other disease-producing strains

of meningococci. The sera could be deficient in antibody to the respective strains of meningococci by lack of previous (or recent) contact with relevant antigens; or as a result of a more general defect in immunoglobulin production such as hypogammaglobulinemia or dysgammaglobulinemia. There could be inhibitors of the bactericidal reaction, abnormalities in complement components, or absence of ancillary factors necessary for the bactericidal reaction. The following experiments were performed to distinguish which of these defects is the one most commonly observed.

Base line sera from cases of meningococcal meningitis are deficient in antibodies to pathogenic strains of meningococci. This was shown by an experiment in which antimeningococcal antibodies (IgG) to prototype strain C11 (serogroup C) were measured by indirect immunofluorescence. Antibodies to strain C11 were not found among 20 of 23 base line sera from cases of group C meningitis. 21 of these sera lacked bactericidal activity to C11 (Table II). However, 23 bactericidal sera from controls each had detectable antimeningococcal antibodies to strain C11 (immunofluorescent titer 1:2 or greater).

Three cases in Table II had bactericidal activity in their base line sera to their own meningococcal isolates. The cause of this lethal activity is not known. No immunoglobulins from classes G, M, or A were found by immunofluorescence to be directed against the homologous meningococcal strains. In contrast, of 10 control sera having bactericidal activity to these meningococcal strains, all had antimeningococcal antibodies (10, IgG; 6, IgM; and 3, IgA).

Base line sera from 23 cases had an average of 169.5 $C'H_{50}$ units/ml (range 162.2 to 186.5 units) control sera 176.8 $C'H_{50}$ units/ml (range 157.9 to 189.4 units), both within the established normal range for the procedure (34).

Sera from susceptible individuals do not appear to lack factors other than antibody which are necessary for bactericidal activity against meningococci, nor do such sera contain inhibitors of the bactericidal reaction (e.g., blocking antibodies, anticomplementary substances). As seen in Table II, 21 of 23 base line sera from cases of meningitis were bactericidal to meningococcal strain G-2-81. Further, such sera were fully able to restore the ability of purified gamma globulin to kill pathogenic strains of meningococci. Table III records the results of an experiment in which a standard amount of base line serum was added in lieu of complement to serially diluted pooled human gamma globulin (Cohn fraction II; 10 mg/ml). In seven of eight instances, the bactericidal titers against the prototype C strain were essentially the same whether base line serum from cases or the standard complement source was used to supply the effector mechanism for bactericidal activity. In the one exception (No. 7123), the complement titer was below normal.

The data indicate that individuals who are susceptible to systemic meningococcal disease lack humoral antibody to the offending strain of meningococcus (as well as to other case strains). However, the results in Table IV show that

such individuals are capable of responding immunologically during the course of infection. Bactericidal titers against homologous strains of meningococci were determined in convalescent sera obtained from 11 cases of meningitis which occurred at Fort Dix, N. J., in 1968. The sera were drawn at least 1 wk after cessation of antibiotic therapy (usually potassium penicillin). In each instance, there was a marked increase in bactericidal activity, reciprocal titers ranging from 256 to 2048. Base line serum from these cases had titers of less than 1:4 (Table II).

TABLE III

Reconstitution of Bactericidal Activity of Human Gamma Globulin by Sera from Prospective Cases of Meningococcal Disease*

Sera		Reciprocal bactericidal titer‡	
Description	Code No.	Serum	Serum (1:4) + gamma globulin§
Base line sera from prospective cases of meningococcal disease	2,651	<4	256
	6,884	<4	128
	7,123	<4	32
	8,860	<4	256
	9,587	<4	128
	10,325	<4	128
	10,662	<4	128
	12,059	<4	128
Complement (P. M.)		<4	128

* Base line sera from recruits in 1st wk of training at Fort Dix, N. J., 1967–1968.

‡ Tested against meningococcal strain C11 (serogroup C).

§ Cohn fraction II (10% w/v) diluted serially in twofold steps. Serum added to final dilution of 1:4. Gamma globulin had no bactericidal activity by itself.

The rise in bactericidal activity in convalescent serum is reflected by the appearance of IgG, IgM, and IgA antibodies to the patient's own strain of meningococcus. Immunoglobulin titers were measured by indirect immunofluorescence in acute and convalescent sera from four cases of meningitis. As shown in Table V, there was a marked, specific rise in level of the three classes of immunoglobulin within 2 wk of onset of disease. It is of interest that there were no detectable antibodies to the homologous meningococcal strains in acute sera from these four cases.

Fate of Susceptible Host Population.—While it is evident that base line sera from cases of meningococcal disease lack bactericidal antibody to the causative organism, it is also true that serum from as many as 35% of young adult males is unable to kill disease-producing strains of meningococci. Why, then, is the attack rate of meningococcal disease so low (less than 1%), even under

epidemic conditions such as existed at Fort Dix in the winter of 1967–68? Three possibilities exist, none of which are mutually exclusive: (*a*) The majority of susceptibles are not exposed to meningococci of proved pathogenicity; (*b*) factors other than circulating antibody predominate in defense against systemic meningococcal disease; (*c*) subclinical cases of meningococcemia occur.

An intensive study of three basic training companies at Fort Dix, N. J., in 1968 provides information on these points. The object was to determine the number of men in each training group who acquired pathogenic strains of men-

TABLE IV

*Bactericidal Activity of Convalescent Sera from Cases of Meningococcal Disease**

Sera		Onset of disease	Reciprocal bactericidal titer‡
Code No.	Date obtained		
A-612	12 April	11 March	1024
A-616	4 April	9 March	512
A-635	23 April	25 March	512
A-637	20 April	22 March	1024
A-640	28 March	28 February	512
A-645	5 April	8 March	2048
A-651	9 April	12 March	1024
A-656	4 April	25 February	1024
A-668	12 April	12 March	1024
A-670	17 April	18 March	512
A-677	6 April	13 March	256

* Fort Dix, N. J., 1968. All cases caused by group C meningococci.

‡ Serum from each patient was tested against his own meningococcal isolate. Exogenous complement was added. Titers of base line sera to the homologous strains were less than 1:4 (Table II).

ingococci against which they lacked serum bactericidal activity. Summarized data from this study are presented in Table VI.

492 men from the three companies were bled and had nasopharyngeal cultures taken during the 1st, 3rd, 5th, and 7th wk of basic training. Five proved cases of meningococcal meningitis, all due to sulfonamide-resistant, serogroup C organisms, occurred during the study period; three in E-5-3 Company (March), one in E-5-3 Company (April), and one in E-2-3 Company (April). The two E-5-3 companies were completely independent training companies. One additional case of meningitis in E-2-3 Company (April) was suspected to be meningococcal in origin, but was not confirmed bacteriologically.

Bactericidal activity of the 1st wk serum from each recruit was tested against the meningococcal strain(s) isolated from the case(s) within his training company. Results in Table VI show that 54 of 492 (11%) recruits had bactericidal titers less than 1:4 against the case strains. This group is defined as the suscepti-

TABLE V

Antimeningococcal Antibodies of Immunoglobulin Classes G, M, and A in Sera from Recruits with Meningococcal Disease‡*

Sera			Reciprocal immunofluorescence titer§		
Patient	Code No.	Date obtained	IgG	IgM	IgA
J. H.	A-199	11/19/66‖	<2	<2	<2
	A-200	11/25/66	128	64	32
C. C.	A-321	1/16/67‖	<2	<2	<2
	A-322	1/23/67	128	128	64
C. S.	A-420	3/16/67‖	<2	<2	<2
	A-421	3/24/67	64	64	32
S. C.	A-428	4/21/67‖	<2	<2	<2
	A-430	4/28/67	512	512	128

* Determined by indirect immunofluorescence.

‡ Fort Dix, N. J. All cases caused by group C meningococci.

§ Sera tested against the patient's own meningococcal isolate.

‖ Date of onset of disease.

TABLE VI

Incidence of Meningococcal Disease among Susceptible Recruits at Fort Dix, N. J., 1968

Training Company	Sera lacking bactericidal activity to case strains*	Susceptible population‡			
		Meningococcal isolates§		Bactericidal activity to acquired C_i strain	Incidence of meningococcal disease
		No. positive/total	No. serogroup C		
	No. negative/total			*No. positive/total*	*No. cases/No. exposed susceptibles (%)*
E-5-3 (March)	30/195	28/30	16	8/16	3/8 (37.5)
E-5-3 (April)	13/185	8/13	3	1/3	1/2 (50.0)
E-2-3 (April)	11/112	8/11	5	2/5	1/3‖(33.3)
Total.....	54/492	44/54	24	11/24	5/13 (38.5)

* All cases caused by sulfa-resistant, group C meningococci.

‡ Individuals lacking serum bactericidal activity to disease-producing strains of meningococci prevalent in their basic training companies.

§ From carriers and cases.

‖ An additional case of meningitis occurred, but the etiological agent was not identified.

ble host population, i.e., the population at highest risk to systemic disease from meningococcal strains of proven pathogenicity within the immediate environment.

44 of the 54 presumed susceptible individuals became carriers of a meningo-

coccus after the 1st wk of training. Of these, 24 (44.4% of the original 54) acquired a sulfonamide-resistant, group C meningococcus. However, serum from 11 of the men had bactericidal activity against the acquired group C strain, indicating that their organisms were different from the case strains. Thus, of the original 54 susceptibles, only 13 were exposed to meningococci compatible with the prevalent pathogenic strains and to which they had no bactericidal activity. The five (and possibly six) cases of meningitis occurred among this group—an incidence of 38.5%.

DISCUSSION

Susceptibility to meningococcal disease in man is related to a selective deficiency of antibody to the offending organism. This conclusion derives from two experiments in which susceptibility to infection and bactericidal activity of serum were compared. In the first experiment, it was found that the age-specific incidence of meningococcal disease in the general population of the United States is inversely proportional to the prevalence of antimeningococcal bactericidal antibodies in the serum. In the second experiment, a study was initiated among military recruits to measure the bactericidal activity of sera from prospective cases of meningococcal disease. The results show that base line sera from cases lack antibodies to the homologous meningococcal strains. Such serum is not only deficient in antibodies to the patient's own strain, but to heterologous disease-producing strains as well—suggesting a lack of immunity to pathogenic meningococci in general. The lack of antimeningococcal antibodies in base line sera from cases was confirmed by indirect immunofluorescence.

3 of 54 sera from prospective cases of meningitis *did* killl homologous and heterologous strains of meningococci. However, neither gamma G, M, nor A antimeningococcal antibodies were present as judged by immunofluorescence. The cause of the bactericidal activity in these three sera is thus unknown.

The deficiency in sera from susceptible hosts is confined to antibodies to meningococci. Hemolytic complement levels are normal, and addition of exogenous complement does not increase bactericidal activity.[1] The sera are fully able to effect bactericidal reactions against meningococcal case strains when reconstituted with purified human gamma globulin (Cohn fraction II). Furthermore, unsupplemented base line sera could kill a group Y strain of meningococcus which was isolated from an asymptomatic carrier.

While sera from susceptible hosts lack antibody to the offending meningococcus, such individuals are capable of initiating an immune response to meningococcal antigens. Thus, convalescent sera from cases of meningitis have markedly increased contents of IgG, IgM, and IgA antibodies to their own meningococcal strain. This increase in antibody titer is accompanied by a sharp rise in bactericidal activity. That the deficiency of antimeningococcal immunoglobulins in sera of susceptibles is not part of a generalized immunological deficiency syndrome is shown by normal total immunoglobulin concentrations in acute

sera from cases (48) and by the absence of a clinical history of unusual susceptibility to other infectious diseases.

Two previous studies have suggested that the prevalence of antimeningococcal antibodies in blood from normal individuals increases as a function of age of the donor. Matsunami and Kolmer (19) titrated the bactericidal activity of whole blood from 26 children, ages 6 months to 10 yr, and from normal adults. They concluded that the blood from children was "somewhat less" meningococcocidal than that from adults. Silverthorne and Fraser (49) also compared bactericidal activity of blood from children and adults. 23 of 33 blood samples from adults were positive against a pathogenic strain of meningococcus, compared to 0 of 11 samples from children less than 2 yr old. Both of these studies suggested that children several months to 2 yr of age lack serum bactericidal antibodies to pathogenic meningococci, whereas most adults have such antibodies. This is consonant with the findings of the present study. In addition, these earlier experiments suggested that young children also lack opsonizing antibodies to meningococci.

There is considerable precedent in the literature of infectious diseases for relating susceptibility to systemic disease to absence of humoral antibodies. Many of the so-called "diseases of childhood" have a reciprocal relationship between the curves formed by age-related incidence of infection and presence of antibacterial or antitoxic antibodies in serum (13). Perhaps the most striking example is that of meningitis caused by *Haemophilus influenzae*. Fothergill and Wright (50) found an inverse relationship between incidence of *H. influenzae* meningitis and bactericidal power of blood almost identical with that shown for *N. meningitidis* in the present study. Not only did the percentage of individuals with sera capable of killing the organism increase progressively after age 3 yr, but the mean bactericidal potency of their sera also increased. Other diseases of childhood in which the development of immunity has been shown to be directly related to a progressive increase in prevalence of specific humoral antibodies with age are diphtheria (51), scarlet fever (52), poliomyelitis (53), and mumps (54).

The strongest evidence that antimeningococcal antibodies in "normal" serum are protective against systemic meningococcal disease is the observation that meningococcal meningitis is unusual in the neonatal period (39, 55, 56) but increases with the onset of physiological hypogammaglobulinemia (57). Analogy with the diseases of childhood cited above suggests that this is due to the transient protective effect of passively transferred maternal antibody (58). The successful use of hyperimmune animal serum in the treatment of systemic meningococcal disease (59) supports the thesis that antimeningococcal antibodies in serum of normal human beings are protective. Bactericidal and opsonic antibodies (60) were present in these therapeutic sera; such antibodies have been described in normal human sera (19–22).

Work in nonimmunized animals also suggests a protective role for "natural" humoral antibodies against meningococci. Matsunami and Kolmer (19), using a single strain of meningococcus, showed that the relative abilities of several types of laboratory animals to survive challenges with graded doses of bacteria correlated with the bactericidal titers of whole blood against the organisms. Recently, Evans et al.[3]

[3] Evans, J. R. Personal Communication.

demonstrated a positive correlation between the abilities of numerous group B strains of meningococci to survive in normal rat serum in vitro and their abilities to cause disease in the rat in vivo.

It is important to emphasize that results of the present experiments are not interpreted to indicate that serum bactericidal activity per se is the protective factor in natural immunity against meningococcal disease. The serum bactericidal test was used only as a sensitive indicator of specific antibodies to meningococci. Such antibodies may have other functions in addition to bactericidal activity (e.g., opsonization); or other, nonbactericidal, antibodies may play a role. Indeed, there is no evidence that humoral antibodies are the sole, or even the major host defense mechanism. Phagocytic cells of the reticuloendothelial system, for example, are undoubtedly of importance in confining and eliminating the meningococcus, and local factors in the nasopharynx may also contribute. Nevertheless, it does seem clear from the present experiments that a deficiency of circulating antimeningococcal antibodies is firmly associated with the establishment of meningococcemia. Epidemiological observations support this conclusion. Thus, while people with humoral antibodies against meningococci frequently become carriers of the organisms,[1] they rarely become cases (6). On the other hand, people who become cases rarely harbor the meningococcus in the nasopharynx more than a few days prior to onset of systemic disease (6, 61). This is presumably because, in susceptible individuals, invasion of the deeper tissues and blood occurs before the carrier state can be established. The relation between the factors that control the carrier state and humoral protective antibodies is not known. However, a later report in this series (62) shows that systemic immunization with meningococcal products can affect local defenses to the meningococcus.

It has been established that meningococcal meningitis is a disease which is spread by asymptomatic carriers (1, 2). Numerous studies have suggested that the incidence of clinical disease is related to the introduction and spread of pathogenic strains of meningococci in the population (1, 7). Our studies suggested that, even during an epidemic, meningococcal disease occurs in only a fraction of the predicted susceptibles. An attempt was made, therefore, to determine the fate of the individuals comprising the susceptible population during an epidemic of group C meningitis at Fort Dix, N.J.

In a group of 492 basic recruits in three training companies, 54 were found to lack serum bactericidal activity against the prevalent disease-producing strains. 44 of this presumed susceptible group acquired a meningococcus during the 8 wk of training, but in only 13 instances were the organisms compatible with the case strains. Of the 13 exposed susceptibles, 5 developed systemic meningococcal disease—an incidence of 38.5% of susceptibles, but only 1% of the total population. These findings indicate that the majority of susceptible individuals

escaped clinical infection because they were not exposed to the prevalent strains of pathogenic meningococci.

It is not surprising that all the potentially susceptible individuals did not become cases upon exposure to the pathogenic strains of meningococci. The fact that base line sera from cases of meningitis have bactericidal titers of less than 1:4 does not mean that all people with titers below this level are susceptible to infection, although many obviously are. Furthermore, other factors, such as intensity of exposure to pathogenic organisms, may be more instrumental in determining the incidence of diseases than exposure per se. Finally, it is possible that in some instances subclinical meningococcemia occurred and was not detected.

SUMMARY

Susceptibility to systemic meningococcal disease is related to a selective deficiency of humoral antibodies to pathogenic strains of meningococci. In a study of the age-specific incidence of meningococcal meningitis in the United States, it was found that the proportion of individuals with serum bactericidal activity to meningococci of serogroups A, B, and C was reciprocally related to the incidence of disease. The prevalence of bactericidal activity was highest at birth and among adults, and lowest in infants between 6 and 24 months of age.

Sera from 51 of 54 prospective cases of meningococcal disease among military recruits were deficient in antibodies to homologous and heterologous strains of pathogenic meningococci as determined by serum bactericidal activity and indirect immunofluorescence. Such sera, however, could support the bactericidal activity of purified human gamma globulin (Cohn fraction II), and such individuals could respond immunologically to infection with meningococci. The implication is that susceptible persons are deficient in antimeningococcal antibodies because they have not received significant exposure to meningococcal antigens in the past.

The fate of individuals who lack bactericidal antibodies to pathogenic meningococci was determined during an outbreak of group C meningitis among military recruits. The incidence of disease was found to be primarily associated with the incidence of exposure of susceptibles to the pathogenic strains. Whereas 81.5% of the presumed susceptibles acquired a meningococcal strain, only 24.1% acquired an organism similar to the prevalent disease-producing strains. Of the exposed susceptibles, 38.5% developed systemic meningococcal disease.

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