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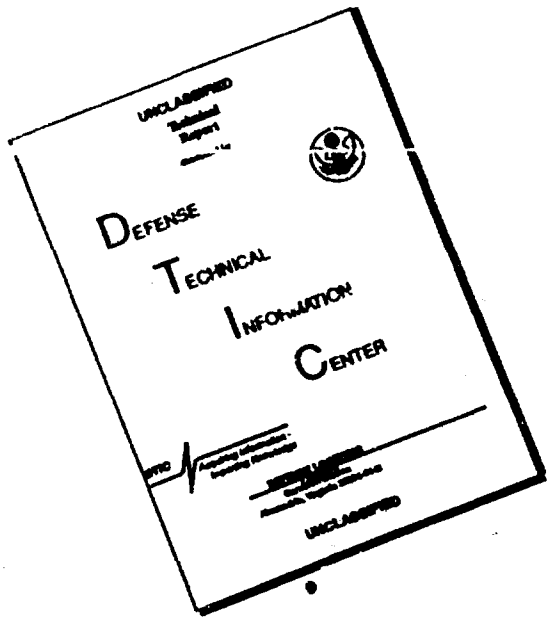

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A Genus Specific Complement Fixation Antigen from *Neisseria meningitidis** (33221)

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The serological diagnosis of meningococcal meningitis has been studied by a number of workers during the past 60 years using the precipitin test (1), bactericidal and agglutination test (2), and opsonin test (3). Additional epidemiological information on meningococcal meningitis has been obtained by fermentation and agglutination reactions of isolates from the patient and his contacts (4). The complement fixation (CF) test has seldom been used for the diagnosis of meningococcal meningitis. Cruickshank (5) was one of the first to use the CF test for the diagnosis of meningococcal disease. Ross and Stevenson (6) assessed the use of the CF test in diagnosing meningococcal 'aseptic' meningitis. Recently, the cell wall has been used as a CF antigen by

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The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Facilities and Care" prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council.

Sanborn and Vedros (7). The antigens used by these investigators were somewhat group-specific, but lacked broad group specificity.

This report describes a method for producing a potent CF antigen which was easily produced in large quantities, not anticomplementary, and reacted specifically with antisera to *Neisseria* species in high titer. Comparison was also made between seroconversions employing this CF antigen and bacterial isolations from recruits at the Naval Training Center, Great Lakes, Illinois.

Materials and Methods. *Antigen preparation. Neisseria meningitidis* groups A (CL-4),[1] B (16B6),[1] C(PTS-5),[1] and strains Bo[1] (8,9), 29E,[2] B (Scarborough),[3] 135,[2] and Z[2] (10) were used in preparing the CF antigens. One ml of each group of an 8-hour culture grown in Mueller-Hinton broth (Difco) was inoculated separately into 250 ml of Mueller-Hinton broth, incubated for 18 hours at 37°C in a Psycrotherm Incubator-Shaker[4] (170–200 rotations a minute) in normal atmosphere (11). The cultures were then killed with a final concentration of 1% beta propiolactone overnight at 6°C. The supernatant fluid recovered after centrifugation (600*g*) was saturated with ammonium sulfate crystals (510 gm/liter) and allowed to set overnight at 6°C with an occasional shaking. The sediment from each 250 ml of original culture was collected after centrifugation (1000*g*) and resuspended in 40 ml of 0.15 *M* NaCl (saline). This crude material was dialyzed against excess amounts of saline until free of ammonium sulfate (24–48 hours). This material was the CF antigen. Further centrifugation reduced turbidity, but a 4-fold loss in potency occurred. The turbidity of the crude antigen did not interfere with determining the CF end points. Therefore, the crude antigen was used for the data presented in this report. Tests for antigen anticomplementariness and potency were performed by standard procedures (12) adapted to the microtechnique (13). Two exact units of complement were used. The primary incubation was for 18 hours at 6°C.

Recruit sera. Blood was collected from recruits upon entering recruit training, at 5 weeks and at 9 weeks of training. Also, recruits who were admitted to the Naval Hospital, Great Lakes with the clinical diagnosis of meningitis were bled upon admission and at approximately weekly intervals for at least 4 weeks. Nasopharyngeal and spinal fluid cultures for *N. meningitidis* were also made on e[illegible]ecruit upon admission to the hospital. [illegible]ra were separated from clotted blo[illegible]l stored at −20°C until tested.

Hyperimmune rabbit serum. Hyperimmune, group-specific antisera were obtained from Difco *N. meningitidis* group A Lot No. 499231, group B Lot No. 512116 and group C Lot No. 511599. Hyperimmune rabbit sera for *N. meningitidis* strains Bo, 135, 29E, and Z were made in this laboratory using the following procedure: the meningococci were grown in Mueller-Hinton broth as described for making CF antigen. The cells were harvested after 6-hours incubation, washed once in saline, and resuspended in 0.5% formaldehyde in saline. New Zealand white rabbits were inoculated intraperitoneally (i.p.) with 1 ml (10^8 organisms) Monday, Wednesday, and Friday of the first week; at similar intervals with 10^9 organisms during the second and third weeks; permitted to rest one week; then inoculated with 1 ml of 10^9 viable organisms intravenously (i.v.) each week until the rabbits developed a satisfactory antiserum for the slide bacterial agglutination test. Rarely were more than 2 i.v. injections required before a high titered antiserum was obtained. Hyperimmune *N. gonorrhoeae* rabbit serum was supplied by William Peacock, Jr., Veneral Disease Research Laboratory, CDC. Hyperimmune *Hemophilus influenzae* and *Diplococcus pneumoniae* antisera were Lot Nos. 496278 and 497496, respectively, from Difco. Sera from patients with *Salmonella typhi* disease were supplied by Miss Jean Koehler, State

[1] Stock cultures obtained from N. Verdos, LT MSC USN, NAMRU-1, Oakland, California.

[2] Strains supplied by Dr. M. Artenstein, WRAIR, Washington, D. C.

[3] Strain isolated from spinal fluid supplied by one of the authors (LFD).

[4] New Brunswick Scientific Company, New Brunswick, New Jersey.

TABLE I. Complement Fixation Titers[a] of Various Hyperimmune Rabbit *N. meningitidis* Antisera Using Antigen Prepared from Different *N. meningitidis* Groups and Strains.

Immune serum	CF antigen prepared from *N. meningitidis* groups and strains (4 antigen units/unit volume)						
	A	B	C	Bo	29E	135	Z
N. meningitidis group A	128	128	128	64	128	64	128
group B	128	256	128	256	256	256	256
group C	64	64	64	128	64	256	256
strain Bo	64	128	64	128	128	256	256
strain 29E	128	512	128	128	128	512	256
strain Z	64	128	64	64	64	256	256
strain 135	64	64	64	64	32	128	256
N. gonorrhoeae	32	64	32	64	64	32	32
N. catarrhalis	ND[b]	16	16	16	32	32	ND
N. flava	32	16	64	64	64	64	ND
N. flavescein	32	64	64	32	64	64	ND
N. sicca	32	16	32	32	16	32	ND
N. perflava	16	16	64	64	128	64	ND
N. subflava	ND	32	64	32	32	64	ND
D. pneumoniae	4	<4	4	4	8	<4	4
H. influenzae	<4	4	4	<4	8	4	<4
S. typhi (O=1:2530; H=1:80)	4	4	8	4	4	4	8
S. typhi (O=1:640; H=1:5120)	4	4	4	4	4	4	4

[a] The reciprocal of initial dilution of serum showing a 4+ fixation.
[b] Not done.

Laboratory of Hygiene, Madison, Wisconsin. All human and rabbit sera were heated at 56°C just prior to testing.

N. meningitidus *isolation*. Nasopharyngeal cultures were taken with a bent cotton swab and inoculated immediately on Mueller-Hinton medium (Difco) containing 10 μg/ml of ristocetin (Difco) and 25 units/ml of polymyxin B. Culture media were maintained above 30°C in an insulated box during transport to the laboratory. The inoculum was then streaked with a bacteriological loop for isolation of colonies and incubated for 18–24 hours in 5% CO_2 atmosphere at 37°C.[5] Organisms from colonies morphologically resembling *N. meningitidis* were tested by slide agglutination using groups A, B, C, and D rabbit antisera (CDC) and rabbit antisera prepared in this laboratory for the "nontypable" meningococcal strains. All isolates agglutinating as *N. meningitidis* were confirmed as such by the usual sugar fermentations and oxidase tests.

Results. The genus specificity of the CF antigen is shown in Tables I and II. Antigens were extracted from 3 groups and 4 strains (nontypables) of *N. meningitidis*. All antigens fixed complement with 7 *N. meningitidis* antisera and antisera to 7 other species of the Genus *Neisseria*. All titers were within the limitation of error of the test except 2 which were 1-tube dilution outside the acceptable range of error. The antisera to *D. pneumoniae*, *H. influenzae*, *S. typhi* O and H antigens fixed complement at a titer of 1:8 or less.

The sera from 3 recruits who became carriers of *N. meningitidis* strain Bo during recruit training were tested for CF antibody with antigens from each of 5 different groups

[5] Model 1237 Hotpack Incubator, Hotpack Corp., Philadelphia, Penn.

TABLE II. Complement Fixation Titers[a] of Various Preparations of *N. meningitidis* CF Antigens Using Homologous and Heterologous Hyperimmune Rabbit *N. meningitidis* Groups and Strains.

Hyperimmune sera	CF antigen prepared from *N. meningitidis* groups and strains						
	A	B	C	Bo	29E	135	Z
N. meningitidis group A	128	128	128	64	64	64	128
group B	128	256	128	256	256	256	256
group C	64	64	128	128	64	128	128
strain Bo	32	128	64	128	64	128	128
strain 29E	128	512	128	128	512	256	512
strain 135	64	64	64	64	64	128	256
strain Z	64	128	64	64	64	256	512
N. gonorrhoeae	64	256	64	64	512	256	256

[a] The reciprocal of the highest dilution of antigen showing a 4+ fixation in the presence of 4 CF units of antibody.

or strains of *N. meningitidis* (Table III). The titer of each serum against each of 5 antigens were essentially identical. Sera from 4 recruits who had clinical meningitis were tested against 6 CF antigens, and showed the same seroresponse to each of the antigens (Table IV). These data indicate that by the seventh day post-hospitalization for meningococcal disease, a substantial increase in antibody titer is shown with a peak titer occurring by day 21. Two of these cases were due to *N. meningitidis* group C, one was due to strain Bo, and one (No 749) had no isolate. Each antigen was made from a different *N. meningitidis* group or strain.

The correlation of 4-fold or greater increase in titers to CF antigen with acquisition of *N. meningitidis*, as determined by isolation, is shown in Table V. The correlation is highly significant ($p = <.0001$).

Discussion. The appearance of specific antibody during convalescence from meningococcal disease was described by Thomas *et al.* (2) Studies have shown that the acquisition of meningococcus in the nasopharynx is followed by the development of meningococcal antibody (7, 14, 15). These

TABLE III. Comparison of Complement Fixation Serum Antibody Titers[a] of Navy Recruit Carriers of *N. meningitidis* Strain Bo Using 5 Complement Fixing Antigens.

Study recruit no.	Week in training	Sample no.	CF antigens for *N. meningitidis* groups or strains				
			29E	C	B (Searborough)	B (16B6)	Bo
1053	0	1	1:4	<1:4	<1:4	<1:4	<1:4
	5	2	1:4	<1:4	<1:4	<1:4	1:4
	9	3	1:64	1:32	1:32	1:64	1:64
1090	0	1	<1:4	<1:4	<1:4	<1:4	<1:4
	5	2	1:64	1:32	1:64	1:64	1:32
	9	3	1:32	1:16	1:32	1:32	1:16
1132	0	1	<1:4	<1:4	<1:4	<1:4	<1:4
	5	2	<1:4	<1:4	<1:4	<1:4	<1:4
	9	3	1:16	1:16	1:32	1:32	1:16

[a] Serum titers expressed as the reciprocal of the highest dilution giving a 4+ fixation in the presence of 4 units of antigen.

TABLE IV. Comparison of Complement Fixation Serum Antibody Titers[a] in Navy Recruits with Clinical Meningitis, Using 6 Complement Fixation Antigens.

Patient	Sample post-admission day	CF antigens from *N. meningitidis* groups or strains					
		A	B	C	29E	135	Bo
226	0	<	4	4	4	4	4
	10	128	256	128	256	256	128
	19	256	128	128	128	128	128
	31	64	64	128	64	64	64
521	0	<	<4	<4	<4	4	<4
	7	8	16	16	8	8	8
	14	32	64	64	64	64	64
	21	32	64	64	64	32	64
782	0	<	<4	<4	<4	4	<4
	7	32	64	128	64	64	64
	14	32	64	128	64	32	64
	21	32	64	128	32	64	64
749	0	<	<4	<4	<4	<4	<4
	7	4	4	4	4	4	4
	14	64	128	128	128	64	64
	21	32	64	64	64	32	64

[a] Reciprocal of the highest dilution of serum giving a 4+ fixation.

data suggested that more extensive investigation of the meningococcal antibody level of the serum may contribute to the understanding of the epidemiology of meningococcal disease. The CF antigen described in this report seems to possess the sensitivity and the group specificity required of an antigen to be employed in epidemiological studies. This is supported by the results in Tables III and IV. These data indicate that

TABLE V. Comparison of CF *N. meningitidis* Seroconversions[a] to Isolations in a Recruit Company in Training from 6 Sept. to 10 Nov. 1967.[b]

	CF seroconversions	
	Positive	Negative
Isolate	45	10
No isolate	5	10

[a] A 4-fold or greater rise in antibody titer.

[b] Bacterial sampling was made only at the times of reporting to and graduating from recruit training; 64% of the recruits seroconverted between the fifth and ninth week serum sample, indicating acquisition late in training and perhaps not sufficient time for antibody to be produced before the final serum sample was taken.

antibody to *N. meningitidis*, whether as a result of carrier status or overt disease, reacts equally with the CF antigen described in this report, regardless of the *N. meningitidis* group from which the antigen was made. Also, more than 3000 recruit sera have been tested with this antigen and with a battery of 14 bacterial and viral antigens. The serological response to *N. meningitidis* was independent of responses to viruses and bacteria (15). The latter infections were due to adenovirus types 4 and 7, influenza A and B, rhinovirus, *M. pneumoniae* and streptococci.

Complement fixation tests with the antigen described can now complement the group-specific indirect hemagglutination (IHA) test (11). This would permit examining large populations for *N. meningitidis* infection experience without the more complex and laborious support required by meningococcal isolates (15). The CF test would serve as a screening test for incidence of infection and the IHA test would identify those groups that were present.

This approach should assist in answering the questions concerning meningococcal in-

fections and disease posed by Thomas *et al.* (2). These questions related to (a) what extent, and what time, antibodies are produced following meningococcal infection, (b) by what serological tests such antibodies may most consistently be demonstrated, (c) what correlation, if any, exists between the development of antibodies and such factors as the severity of infection, the rapidity of recovery, or the biological characteristics of the infecting organism, and (d) what levels of antibody are to be found in the blood of carriers and normal individuals in an epidemic area.

Summary. A simple method for preparing an *N. meningitidis* complement fixing antigen is described. Hyperimmune rabbit *N. meningitidis* antiserum reacted approximately the same with CF antigens made from several *N. meningitidis* groups or strains. Recruits known to have become infected with *N. meningitidis* strain Bo during recruit training show equal serological responses to CF antigens made from different *N. meningitidis* groups or strains. Further, 4 recruits who were admitted to the hospital with clinical meningitis also seroconverted equally to any 1 of 6 different *N. meningitidis* CF antigens. Increase in CF titer (4-fold or greater) to *N. meningitidis* correlated significantly with acquisition of meningococcus in the nasopharynx as determined by bacterial isolations.

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