PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 289-291 (1976)

Meningococcal Group C Subgroup Determinant Detected by Immunofluorescence[1] (39381)

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Using an hemagglutination inhibition method (HAI) and isolated capsular antigens, a subgroup determinant, C_{1+}, was demonstrated in the polysaccharides of 26 of 30 Group C meningococcal strains (1). Polysaccharides containing the subgroup determinant were resistant to digestion by neuraminidase, 0.1 unit per milligram of antigen, whereas those which lacked the determinant, designated C_{1-}, were rapidly destroyed by the same concentration of enzyme. In this report is described an indirect immunofluorescent antibody test system (IFA) specific for the subgroup determinant and permitting the separation of intact Group C meningococci into C_{1+} and C_{1-} subgroups.

Materials and methods. Cultures. Two collections of Group C meningococcal strains were studied with the IFA subgroup system: (i) 20 strains previously studied with the HAI method (1), and (ii) 196 strains from the collection of one author (HAF). The latter were categorized only by IFA. The sulfadiazine sensitivity patterns of 121 of the 196 strains were determined (2). All strains were preserved lyophilized in Mueller-Hinton broth or in defibrinated rabbit blood at −70°.

Test preparation. Cultures were reconstituted on tryptose blood agar plates and incubated overnight at 35° under 5% CO_2. Bacteria were removed from the agar medium with loops and washed twice in phosphate-buffered saline, pH 7.2 (PBS). They were resuspended in the same diluent at an optical density of 0.20 at 660 nm and approximately 10 lambda of each suspension were placed on glass slides, spread, and air-dried. All were studied on the day that the slides were prepared.

Subgroup test. Antiserum 20 produced in rabbits by immunization with a C_{1+} polysaccharide (3) and previously shown to contain antibodies to the subgroup determinant (1), was used as the unconjugated serum. This antiserum had been exhaustively absorbed with mouse liver powder and Group C meningococcal strains lacking the subgroup determinant by HAI. A sheep-anti-rabbit immunoglobulin antiserum was conjugated with fluorescein by the method of Clark and Sheppard (4); its *f/p* ratio was between 3 and 4. Slides were read on an American Optical series 10 Microstar microscope with a Fluorolume illuminator using 695 (Corning 5113) excitor and Schott OG-1 barrier filters. Positive and negative fluorescent staining were judged independently be two observers. HAI tests specific for Group C and subgroup determinants and neuraminidase digestions were performed as previously described (1). Capsular antigens for use as inhibitors of this system were prepared by the method of Robinson and Apicella (3).

Results. Initial studies with the subgroup IFA system were performed on the 20 Group C meningococcal strains which previously had been analyzed for the subgroup determinant by the HAI method. The 16 cultures which were C_{1+} in this system also were positive by IFA, while the four C_{1-} strains were negative by IFA. Among the second collection of 196 Group C meningococcal strains, 143 (73%) were IFA positive and 48 (24.5%) were negative. The remaining 5 (2.5%) cultures were both subgroup negative and positive. After replating, 10 single colonies were selected

[1] Supported by Grant No. 5-R01-AI-1023604 from the National Institute of Allergy and Infectious Diseases (M.A.A.), and U.S. Army Medical Research and Development Command Contract No. DAMD17-74-C-4008 (H.A.F.). Dr. Apicella is a recipient of USPHS Research Career Development Award No. 5-K04-AI-70747-02.

from each for study by IFA. This time all were either subgroup positive or negative and not mixed as their parent cultures had been. In addition, Group C polysaccharides isolated from negative and positive colonies from the mixed cultures had the C_{1+} determinant only on the IFA positive subculture and not on the IFA negative one.

Further confirmation of the reliability of the IFA method was obtained from the following experiment. Polysaccharides isolated from 5 Group C, C_{1+} and 5 C_{1-} strains were analyzed for the subgroup determinant by HAI and their susceptibilities to neuraminidase determined. All strains identified as C_{1+} by IFA contained the subgroup determinant by HAI and were resistant to digestion by neuraminidase while the 5 C_{1-} strains lacked the determinant and were readily digested by the enzyme (Table 1).

The distribution of C_{1+} and C_{1-} strains appears to be unrelated to geography. Among 147 isolates from civilian and military sources in the continental United States, 110 (75%) were C_{1+} and 37 (25%) were C_{1-}. Of an additional 41 strains from five foreign countries on four continents, 32 (78%) were C_{1+} and 9 (22%) were C_{1-}.

Among the 196 strains, 15 were isolated from the nasopharynges of members of seven families with one or more cases. In these seven families, both case and carrier cultures were C_{1+} except for one family with two cases. The isolate from one child was

TABLE I. NEURAMINIDASE SENSITIVITIES OF POLYSACCHARIDES OF MENINGOCOCCAL GROUP C SUBGROUPS DETECTED BY IFA AND HAI TESTS.

	Subgroup		Serogroup C (HAI)[a]	
Culture number	IFA	HAI (μg/ml)	Before (μg/ml)	After (μg/ml)
88	+	8.0	16.0	4.0
215	+	4.0	4.0	8.0
252	+	8.0	16.0	16.0
54	+	2.0	1.0	2.0
78	+	16.0	4.0	4.0
101	−	>1000[b]	16.0	>1000
194	−	>1000	4.0	>1000
125	−	>1000	2.0	>1000
144	−	>1000	8.0	>1000
53	−	>1000	16.0	>1000

[a] Before and after the digestion of 1 mg of polysaccharide with 0.1 unit neuraminidase for 16 hr.
[b] No inhibition at 1000 μg/ml.

TABLE II. COMPARATIVE FREQUENCIES OF SUBGROUP DETERMINANT IN STRAINS ISOLATED FROM CASES AND CARRIERS.

		Cases[a]		Carriers[b]	
C_1 subgroup	Sample number	Number	%	Number	%
+	132	97	73	35	27
−	34	31	91	3	9
Totals	166	128	77	38	23

[a] Spinal fluid, blood, joint, or eye.
[b] Nasopharynx, throat, sputum, or cervix. ($\chi^2 = 4.795, p = 0.05 > p > 0.025$.)

C_{1+} and from the other was C_{1-}. In three other groupings of two cases each (apparently hospital-related), two pairs were both C_{1-} and the third was C_{1+}. All of these family and hospital groupings were of civilian origin. Multiple isolates from military cases and carriers were C_{1+} as were the carriers in a population of "normal" families (5).

The sites of origin of study strains were available from 164 different individuals (Table II). C_{1-} strains were more often associated with clinical disease than were C_{1+} strains ($\chi^2 = 4.795, p = 0.05 > p > 0.025$). No statistically significant differences were found between susceptibility to sulfadiazine and subgroup type in 121 case strains ($\chi^2 = 2.207, p = 0.25 > p > 0.10$).

Discussion. Two systems for separating Group C meningococci into different subgroups have been reported. Gold and Wyle, using a bactericidal antibody assay directed against noncapsular cell wall factors, defined six serotypes. There seemed to be an association between increased attack rates (virulence) and resistance to sulfadiazine for serotype 2 (6). Counts *et al.* (7) described a typing system for Group B and C meningococci based upon the action of meningocidins. They identified 11 types with the majority being of type 1.

The subgrouping system described in this report utilizes IFA to identify a subgroup determinant present on the C polysaccharide. The results obtained with this system indicate that this determinant geographically is widely distributed. The presence or absence of the determinant and the strain's sulfadiazine sensitivity characteristics are unrelated. Our experience suggests that C_{1-} strains are associated with clinical disease to

a significantly greater degree than are those of C_{1+}. Whether this is related to virulence or reflects a sampling error remains to be determined.

The IFA method for subgroup detection is rapid and may be useful epidemiologically. For the latter, as described herein, it may be of value in defining potential meningococcal contact clusters. Thus, application of the subgroup IFA procedure may help to identify the route of transmission of Group C meningococci in outbreaks and in population carrier surveys. It also may have some value in the selection of strains for vaccine production.

Summary. The presence or absence of a meningococcal Group C subgroup antigen (C_{1+}) can be detected by immunofluorescence as readily as by a previously reported hemagglutination-inhibition method. When compared in strains isolated from cases and carriers, subgroup negative (C_{1-}) strains seemed to occur more often in cases. The subgroup antigen is widely distributed geographically. The characterization of meningococci by its presence or absence may be of value in epidemiological studies.

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Received October 14, 1975, P.S.E.B.M. 1976, Vol. 152.