Serotypes of *Neisseria meningitidis* Isolated from Patients in Norway During the First Six Months of 1978

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During the first 6 months of 1978, 114 strains of *Neisseria meningitidis* isolated from patients in Norway were serotyped. Among 27 group C strains, type 2 was most common, whereas 82% of the 82 group B isolates did not react with antisera to the standard serotypes 1 to 12. These strains were shown to belong to a new serotype, type 15. Also some group A and C strains had the type 15 antigen. Investigations on a possible immunoprophylaxis against group B meningococcal disease in Norway should accordingly proceed with type 15 rather than with type 2 meningococci.

The incidence of meningococcal disease in Norway suddenly increased in 1974 (1) and has remained at about the same high level since. Group B *Neisseria meningitidis* accounts for about three-fourths of the cases. Also, in carriers there is a dominance of group B strains (11, 12).

Serotyping based on outer membrane protein antigens (4, 10) offers more details on the epidemiology of meningococcal disease and has been a valuable tool in epidemiological studies (2, 3, 9, 14). Type 2 has been responsible for at least one half of the cases (2, 3, 6). Antibodies to these protein antigens are also important in the protection against meningococcal disease (3).

In the present study strains of *N. meningitidis* isolated from patients in Norway during the first 6 months of 1978 have been serotyped. The study was done to get more detailed epidemiological data than can be obtained by serogrouping and also because information about the serotype distribution is essential when the possibility of a future immunoprophylaxis against group B meningococcal disease is considered.

MATERIALS AND METHODS

Bacterial strains. A total of 114 strains of *N. meningitidis* isolated during the first 6 months of 1978 were received from all microbiological laboratories in Norway. All strains were isolated from cerebrospinal fluid or blood culture in cases of meningococcal infection. Four strains grew in blood culture as well as in cerebrospinal fluid, 26 grew in blood culture only, 71 grew in cerebrospinal fluid only, and 13 grew in blood culture or cerebrospinal fluid, it is not known which. After primary isolation at the local laboratory, the strains were transported in Stuart medium to Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt for reference serogrouping. The strains were freeze-dried as soon as possible after arrival.

Serotype reference strains. Serotype reference

strains for the meningococcal serotypes 1; 2; 2,7; 2,10; 4; 5; 6; 8; 9; 11; and 12 were kindly provided by Carl E. Frasch, Bureau of Biologics, Bethesda, Md.

Serotype antisera. Serotype antisera for immunodiffusion experiments were made in rabbits by intravenous injections of Formalin-killed meningococci (4). The sera used in the bactericidal experiments were made by inoculating rabbits subcutaneously with serotype antigen partially purified by ultracentrifugation (7). In addition to antisera to the types 1 to 12, antiserum was also made with strain 44/76, which had been nontypable in previous tests (Frasch, personal communication), and which had been isolated from a fatal case of meningococcal septicemia.

Microbactericidal assay and absorption of antisera. Microbactericidal assay and absorption of antisera were done as described by Frasch and Chapman (4).

Serotyping. Serotyping was done by immunodiffusion in agar gel (5). The antigen was extracted with 0.2 M LiCl-0.1 M sodium acetate, pH 5.8, for 2 h at 50°C (7) from cells grown overnight on blood agar plates. Sonic disruption before extraction seemed to increase the amount of antigen released. Later in the study, blood agar-grown cells were extracted with 0.15 M NaCl at 100°C for 15 min (5). Extraction with LiCl was then done only in those few instances when NaCl extraction failed.

Serogrouping. Serogrouping was done by slide agglutination as described elsewhere (13).

RESULTS AND DISCUSSION

New serotype. Preliminary experiments showed a number of strains to be nontypable by antisera to the standard types 1 to 12. Antiserum to one of the nontypable strains, 44/76 (group B), cross-reacted weakly with type 12 in immunodiffusion and bactericidal experiments. Bactericidal tests with cross-absorbed antisera, summarized in Table 1, indicate that strain 44/76 belongs to a serotype not recognized before, and

TABLE 1. Bactericidal activity of cross-absorbed antisera to the meningococcal strains S-3032 (type 12 reference strain) and 44/76

Serum	Strain used for absorption	Bactericidal activity against:	
		S-3032	44/76
Anti-S-3032	— ^a	+ ^b	+
	S-3032	—	—
	44/76	+	—
Anti-44/76	— ^a	—	+
	S-3032	—	+
	44/76	—	—

^a Sera were not absorbed.^b +, Bactericidal activity at titer greater than 1:40; —, no bactericidal activity.

it has been designated type 15, as suggested by Frasch.

Distribution of serotypes. Distribution of serotypes among the 114 strains is shown in Table 2. Elsewhere in the world, type 2 has been the most common one (2, 3, 6). This was the case only among group C strains in the present material. Surprisingly, only 2 of the 82 group B strains were type 2, whereas as many as 89% reacted with antiserum to type 15.

No attempt was made to find out whether the type 2 strains also contained the serotype antigens 7 and 10. Particularly in the type 2-associated strains of *N. meningitidis*, more than one antigen may coexist in the same strain (4). Also, some type 15 strains possess an additional antigen (Table 2).

During the years 1969 and 1970, group A accounted for 27 out of 57 isolated strains of *N. meningitidis* (47%), and 20 strains (35%) were group B. The rest were mainly group C (Holten, unpublished data). Concomitant with the rise of incidence in 1974, there has been a shift towards a dominance of group B meningococci (1), up to the present 72% (Table 2). Group A has almost vanished.

A recent introduction of type 15 meningococci into a susceptible population might have contributed to the present high incidence of the disease. Type 15 strains, however, have been found among meningococci isolated as long ago as 1969, but to what extent this serotype was present at that time remains to be investigated.

Because of the poor immunogenic properties of the group B meningococcal polysaccharide (15), interest has been focused on the serotype protein antigen with respect to a possible immunoprophylaxis against group B meningococcal disease. Antibodies to type 2 antigen are found after nasopharyngeal carriage of type 2 meningococci and after disease due to this se-

TABLE 2. Groups and types of *N. meningitidis* isolated from patients in Norway during the first 6 months of 1968

Serotype	No. isolated from serogroup:			
	A	B	C	Y
1		1		
2 ^a		2	13	1
4			1	
5		1		
6		1		
9	2		1	
11		1		
15	1	67	9	
15,2			1	
15,4		2		
15,8		4	1	
15,9	1			
NT ^b		3	1	

^a No distinction was made between the types 2; 2,7; and 2,10.^b NT, Nontypable.

rotype. Vaccination with the type 2 antigen protects guinea pigs against infection with type 2 meningococci (8), and also evokes a slight immunological response in humans (16). Because most type 2 strains isolated in Norway are group C, the immunogenic properties of the type 2 antigen are not important in this country. More interesting would be the immunological response to carriage of, and to disease due to, type 15 organisms. Studies along these lines are in progress.

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