

Meningococcal Phenotypic and Genotypic Characteristics and Human Antibody Levels

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During a 2-month period in 1984, throat and blood samples were collected from 1,102 healthy persons of different ages living in the city of Tromsø, Norway. One hundred and eight persons (9.8%) were meningococcal carriers, but the carrier rate varied with sex and age. Twenty-nine isolates (26.9%) were of serogroup B, and 31 (28.7%) isolates contained the serotype 15 antigen. Sixty-eight (63.0%) isolates were nontypable, 49 (45.4%) were nongroupable, and 21 (19.4%) were sulfonamide resistant. All nine serotype 2a isolates and eight (25.8%) of the serotype 15 isolates were sulfonamide resistant. Only these eight serotype 15, sulfonamide-resistant isolates had a DNA fingerprint similar to that of the majority of systemic isolates of *Neisseria meningitidis* in Norway. The average level of antimeningococcal immunoglobulin G antibodies, as determined by a whole-bacterium enzyme immunoassay with a systemic B:15 meningococcal strain as the antigen, was low until 12 to 15 years of age and then steadily increased.

For many years *Neisseria meningitidis* has been the most common cause of meningitis and septicemia in children and youths in Norway, with a mortality rate above 10% (2). During the period 1974 to 1986, the yearly incidence of meningococcal disease varied between 6.7 per 100,000 inhabitants (1977) and 8.1 per 100,000 inhabitants (1975). An intriguing question is why colonization of the nasopharynx with meningococci leads to the formation of protective antibodies in some people (10; I. Nessiøy, student diploma, University of Tromsø, Tromsø, Norway, 1983) while others may develop systemic disease. To understand the pathogenicity and epidemiology of meningococcal disease, it is necessary to characterize carrier strains phenotypically and genomically, to study their distribution in the normal population, and to know the immunity against meningococcal disease in different population groups. Most studies on the carriage of meningococci have been performed on selected population groups such as soldiers and contacts of cases with meningococcal disease, or the material has been collected over several years (6, 8, 13, 14).

The aim of the present investigation was to study the meningococcal carrier rate and the levels of antimeningococcal immunoglobulin G (IgG) antibodies in a healthy population, to characterize the carrier isolates by serogrouping, serotyping, and sulfonamide susceptibility testing, and finally to determine the frequency of the predominant disease-associated strain of meningococci in a healthy population in Norway by DNA fingerprinting of chromosomal DNA (1, 15-18).

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MATERIALS AND METHODS

Tromsø has 45,000 inhabitants and is the largest city in the county of Troms, North Norway. During the period 25 May to 10 July 1984, throat and blood samples were collected from 1,102 Tromsø citizens of all ages who had no clinical signs of infection. Five cases with systemic meningococcal disease were reported in the city of Tromsø in 1984, all of them before 20 April. Samples from children and youths were collected at six welfare centers, nine schools, and nine day care centers evenly distributed throughout the community. Samples from adults were collected at various places of work, at the hospital out-patient clinic, at the office of a general practitioner, and at a day care center for the elderly. Table 1 shows the number of males and females sampled in nine different age groups.

Blood samples. Capillary blood was collected by puncture of the fingertip with an automatic lancet (Autolet; Owen Mumford, Woodstock, England). Serum was obtained by immediate centrifugation of the blood at $6,000 \times g$ in small tubes with a barrier material in the bottom (Microtainer system; Becton Dickinson, Rutherford, N.J.). The sera were kept frozen at -22°C .

Throat cultures. Throat samples were obtained from the tonsils and posterior pharynx with a charcoal-impregnated swab and plated immediately on GC agar base supplemented with hemin and IsoVitalX (BBL Microbiology Systems, Cockeysville, Md.) and containing colistin and vancomycin. The plates were incubated as described previously (17). Meningococci were identified by conventional procedures (19).

Serogrouping. Meningococci were primarily serogrouped by slide agglutination with commercially available antisera (Wellcome Reagents Ltd., Beckenham, England) and a group 29E serum kindly provided by I. Lind, Statens Seruminstitut, Copenhagen, Denmark. Polyagglutinating strains were further tested by combined gas chromatography and counterimmunoelectrophoresis as described previously (3, 4, 18).

TABLE 1. Age and sex distribution of study population

Sex	No. in age group									Total no.
	0-3 yr	4-7 yr	8-11 yr	12-15 yr	16-19 yr	20-29 yr	30-39 yr	40-59 yr	≥60 yr	
Female	47	89	67	87	81	51	34	48	52	556
Male	57	66	61	105	79	42	36	38	62	546

Serotyping. Serotyping was performed as described previously (18) with monoclonal antisera against serotypes 2a, 2b, and 15 and subtypes P1.2 and P1.16 (kindly provided by W. D. Zollinger, Walter Reed Army Institute of Research, Washington, D.C.).

Sulfonamide susceptibility testing. MICs of sulfadiazine were determined by the agar dilution method (3, 18). Isolates for which MICs were 5 µg/ml or lower were considered sulfonamide susceptible, and those for which MICs were 100 µg/ml or higher were considered resistant. Isolates for which MICs were between 5 and 100 µg/ml were scored as intermediate.

DNA fingerprinting. DNA fingerprinting of chromosomal DNA was performed as described previously (18). The individual band patterns were then compared with the DNA fingerprint of the systemic isolate O35 (serogroup B, serotype 15, and sulfonamide resistant), which was shown previously to possess a DNA fingerprint similar to that shared by most (78.7%) systemic meningococcal isolates in Norway (Sørensen et al., unpublished results).

Serology. Antimeningococcal IgG antibodies were quantitated within 2 months after collection of serum by a whole-bacterium enzyme-linked immunosorbent assay (ELISA) technique with a systemic B:15 isolate (8065/75) as the antigen and with alkaline phosphatase-labeled anti-human IgG as the conjugate (17). Pooled normal human serum absorbed with the antigen strain 8065/75 was used as the negative control for IgG quantitation. The convalescent-phase serum of a patient recovered from meningococcal disease was used as a positive control. The cut-off value was defined as the mean optical density (OD) of three replicates of the negative controls plus 3 standard deviations. The results are given as the geometric mean of the OD above the cut-off for each age group.

RESULTS

Carrier rate. Of the 1,102 persons included in the study, 108 (9.8%) harbored meningococci in their throat. The

carrier rate varied with age and sex (Fig. 1). The carrier rate started to increase from early childhood, peaking for males in the age group 30 to 39 years (25.2%) and in females at 16 to 19 years (18.5%). Thereafter, a steady decrease in meningococcal carrier rate was observed. The lowest carrier rate in both females (1.9%) and males (6.5%) was found at ages above 60 years. The carrier rate was higher in males (11.4%) than in females (7.9%), but the difference was only significant ($P < 0.01$, chi-square test) in the group of 20 to 29 years of age.

Bacterial phenotypic characteristics. Table 2 shows the serogroup and serotype distribution of the 108 meningococcal isolates. Most isolates were groupable (54.6%), and 26.9% were of serogroup B. Sixty-eight (63.0%) isolates were nontypable, of which 12 reacted with the subtype P1.16 or P1.2 antiserum. Thirty-one isolates (28.7%) reacted with the serotype 15 and nine (8.3%) with the serotype 2a antisera. A total of 21 (19.4%) isolates were sulfonamide resistant, 8 of them containing the serotype 15 antigen and 9 of them the serotype 2a antigen.

DNA fingerprint analysis. Figure 2 shows the DNA fingerprints of different carrier isolates. Of the 108 isolates, 8 (7.4%) had a DNA fingerprint similar to that of strain O35. This DNA fingerprint was associated with the simultaneous presence of the sulfonamide resistance and the serotype 15 characteristics (Table 3). All isolates with these characteristics had this particular DNA fingerprint, and this DNA fingerprint could only be found in these eight isolates. Of the 13 isolates that were sulfonamide resistant but lacked this particular DNA fingerprint, 9 were of serotype 2a.

Antimeningococcal IgG antibodies. The level of antimeningococcal IgG antibodies varied with age (Fig. 3). Very low levels were present at ages below 12 years but then the levels started to increase and increased steadily until the oldest age studied. Above 20 years of age, the levels of antimeningococcal IgG antibodies were significantly higher in males than in females ($P < 0.001$, Wilcoxon nonparametric two-sample test). No clearcut correlation was observed

TABLE 2. Serogroups, serotypes, sulfonamide resistance, and restriction endonuclease band pattern of 108 meningococcal carrier isolates

Characteristic	No. of isolates of serotype:subtype ^a :						Total no. (% of isolates)
	15:—	15:P1.16	2a:—	2a:P1.2	NT:P1.16	NT:P1.2	
Serogroup							
A							1 (0.9)
B	9	4			4	1	29 (26.9)
C	1		1	6		1	13 (12.0)
X							1 (0.9)
Y							3 (2.8)
Z							0
W135							3 (2.8)
29E					1		8 (8.3)
NG ^b	12	5		2	2	3	25 (45.4)
Sulfonamide resistance	2	6	1	8	0	1	21 (19.4)
O35 band pattern ^c	2	6					

^a NT, Nontypable.

^b NG, Nongroupable.

^c Restriction endonuclease band pattern similar to that of the systemic isolate O35 (B:15, sulfonamide resistant; see text).

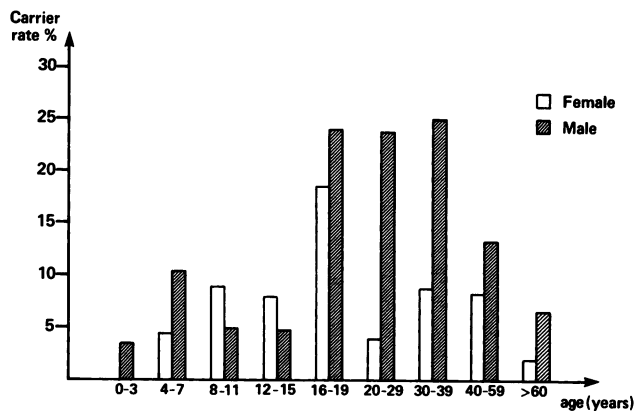


FIG. 1. Carrier rate of *N. meningitidis* in 1,102 healthy persons in Tromsø, Norway.

between carrier rate and levels of antimeningococcal IgG antibodies for different age groups. The level of antimeningococcal IgG antibodies in different age groups was plotted against the age-related incidence of meningococcal disease in the county of Troms between 1979 and 1982 (86 cases [Nessiøy, diploma]). Figure 4 shows that the incidence was highest in the youngest age group, where the level of antimeningococcal IgG antibodies was lowest.

DISCUSSION

In the present study specimens were collected during a 2-month period. Even though the disease is endemic in North Norway, no cases of meningococcal disease occurred within a month of the sampling period. In our study, we found an overall meningococcal carrier rate of 9.8% in the general population. In previous studies in Norway, carrier rates of 43.4% in military recruits (13) and of 39.0% in a semi-isolated population (coal miners and their families) were found 3 weeks after a case of meningococcal disease occurred (14). The lower carrier rate in our study is probably due to differences in the populations studied, because it has

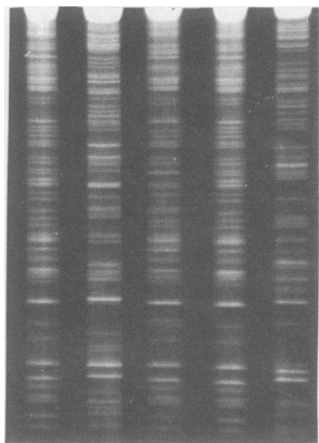


FIG. 2. DNA fingerprints of the systemic isolate O35 (B:15, sulfonamide resistant; see text) and of different carrier isolates. From the left are shown O35, BT888 (B:NT:—, sulfonamide susceptible), BT895 (B:15:—, sulfonamide resistant), O35, and BT900 (C:2a:—, sulfonamide resistant). BT895 has a DNA fingerprint similar to that of O35.

TABLE 3. Phenotypic properties of meningococcal carrier isolates with a DNA fingerprint similar to that of most Norwegian systemic isolates^a

Isolate no.	Serogroup	Serotype:subtype
BT41	NG ^b	15:—
BT146	NG	15:P1.16
BT147	B	15:P1.16
BT294	B	15:P1.16
BT325	NG	15:P1.16
BT798	B	15:P1.16
BT895	B	15:—
BT930	NG	15:P1.16

^a All the isolates were sulfonamide resistant.

^b NG, Nongroupable.

been shown that military recruits and contacts of cases with meningococcal disease have a higher meningococcal carrier rate than the general population (5, 21).

In both sexes (Fig. 1), the carrier rates were highest between 15 and 39 years of age, which agrees with previous studies (8, 9). The carrier rate was significantly higher in males than in females in the group aged from 20 to 29 years. The explanation of this difference may be that women at this age tend to stay at home with their children and have fewer social contacts than their menfolk and that many males in this age group have recently left the military, where the carrier rate is much higher than among civilians.

Systemic isolates from Norway are phenotypically very homogeneous, more than 80% of them being sulfonamide resistant, serogroup B, and serotype 15 and belonging to a group of genetically related clones (3, 5, 16). In the present study, we show that carrier isolates are phenotypically heterogeneous. In agreement with other studies (6, 7), the carrier isolates were predominantly nongroupable (45.4%) or nontypable (63.0%). In contrast to systemic isolates, relatively few (19.4%) of the carrier isolates were sulfonamide resistant. Sulfonamide resistance was concentrated in isolates of serotype 2a (9 of 9) and serotype 15 (8 of 31). Among the systemic isolates, however, 98% of the serotype 15 isolates were sulfonamide resistant (3).

DNA fingerprint analysis showed that eight (7.4%) of the carrier isolates had a DNA fingerprint similar to that of the epidemic strain in Norway, represented in our study by isolate O35. All eight isolates were sulfonamide resistant,

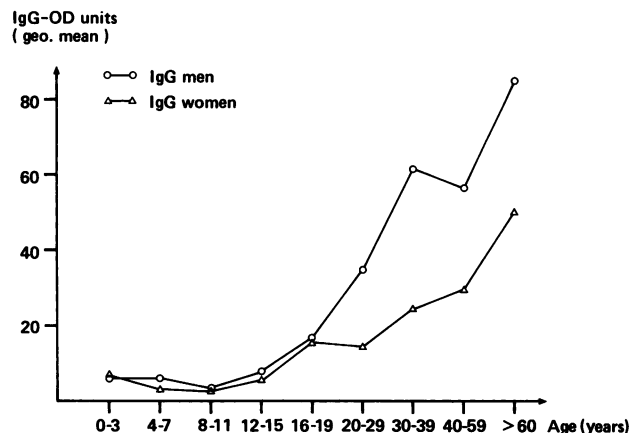


FIG. 3. Antimeningococcal IgG antibodies related to age and sex for 1,102 healthy persons in Tromsø.

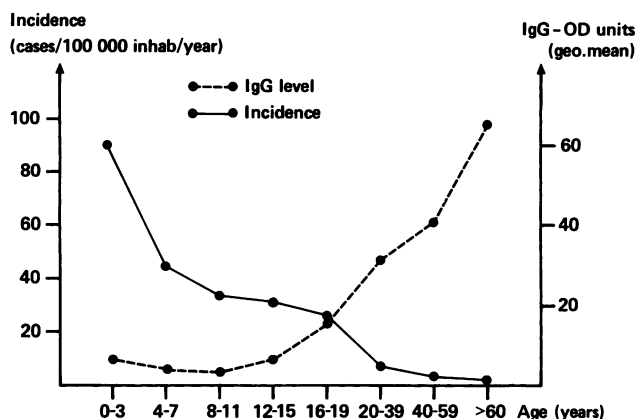


FIG. 4. Antimeningococcal IgG levels of 1,102 healthy individuals in Tromsø and the incidence of meningococcal disease in the county of Troms (cases per 100,000 inhabitants per year) between 1979 and 1982 related to age.

and all contained the serotype 15 antigen. However, they differed in serogroup in that four of them were nongroupable and four were of serogroup B. The reason for this discrepancy may be that meningococci are able to vary their expression of capsular polysaccharide (18). On the basis of these results, we estimate the prevalence of the epidemic strain in the population of Troms to be 0.7%. In Finland, the epidemic strain was present in 0 to 3% of the civilian population (21).

Our results show that the mere presence of a meningococcus of the pathogenic genotype in the throat is not in itself sufficient to cause disease. However, healthy persons colonized with pathogenic meningococci may be the source of outbreaks of systemic meningococcal disease. If such potentially pathogenic bacteria could be rapidly identified and epidemiological control measures initiated, cases of meningococcal disease could be prevented. We believe that the epidemic strain can be identified by DNA fingerprinting or by serotyping combined with sulfonamide susceptibility testing. Because these methods are time-consuming and labor intensive, a more rapid method for strain identification is required. A DNA probe that is specific for the epidemic strain and that can be used in a dot-blot assay would fulfill this requirement. Work along this line is in progress.

It has been shown that protection against meningococcal disease is correlated to bactericidal antibodies (10) and that bactericidal antibodies in the convalescent-phase sera of patients recovered from serogroup B meningococcal disease are directed mainly against outer membrane antigens (11). Harthug et al. (12) used an outer membrane antigen ELISA test to diagnose meningococcal disease. Because the bactericidal antibody test is difficult to standardize and time-consuming and because the anti-outer membrane antigen antibodies have not yet been shown to be protective against meningococcal disease, we used a whole-bacterium ELISA to measure population immunity against meningococcal disease (17). Our results show that the level of antimeningococcal IgG antibodies is low in the population below 12 to 15 years of age. From then on a steady increase in IgG antibodies occurs, and in both males and females the highest level was observed in the oldest age group studied. The reason for this increase in IgG antibodies throughout life may be stimulation of the immune system during transient carriage of or subclinical infections with meningococci or other neisseriae and bacteria that have been shown to induce

cross-reactive antibodies (8, 10). Plotting the incidence of meningococcal disease in different age groups against the corresponding level of IgG antibodies in the population showed an inverse relationship between these two parameters, as was also found by Goldschneider et al. (10). In the younger age groups with low levels of antibodies, the incidence of meningococcal disease is highest. However, the incidence starts to decline before any detectable increase in the antimeningococcal IgG antibody level. The reason may be that the ELISA is not sensitive enough for quantitation of protective antibodies. The test measures a large number of antigen-antibody interactions, but only a small number of them may confer protection.

In spite of extensive research during the last two decades, the epidemiology and pathogenicity of meningococcal disease remain obscure. A better understanding of the meningococcal carrier state and of population immunity as well as studies of meningococcal carrier isolates are required for progress in the prevention of meningococcal disease.

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