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Strains of *Neisseria meningitidis* Isolated from Patients and Their Close Contacts

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Neisseria meningitidis isolates from contacts, mostly family members, of 27 unrelated meningococcal disease patients were examined by serogrouping, serotyping, and a recently described sodium dodecyl sulfate-polyacrylamide gel electrophoresis typing procedure. Most of the isolates were serogroup B or C. Serotyping and sodium dodecyl sulfate-polyacrylamide gel electrophoresis typing now provide a more precise means than serogrouping for determining the epidemiological relationships among patient isolates and those of related carriers. In 70% of the families studied, all contact carriers had strains indistinguishable from that of the patient. In the other 30%, more than one meningococcal strain was recovered from the family. Sixty percent of the carrier isolates were recovered from adults. It was found that, among household contacts, the mother was most likely and the father was least likely to carry the disease isolate. Nonhousehold contacts were least likely to carry the disease isolate.

Little is known about the spread of individual *Neisseria meningitidis* strains within families and communities. Epidemiological studies of meningococcal infections have been hampered by the inability to distinguish strains within serogroups. The development of serotyping and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) typing methods have permitted the identification of individual meningococcal strains (9, 24). Whereas serogroups are identified on the basis of immunologically distinct capsular polysaccharides (4), serotypes are identified by immunologically distinct outer membrane proteins and lipopolysaccharides (8, 24). Some strains, especially those isolated from healthy carriers, cannot be typed with the existing protein serotype reagents (5, 8) and are referred to as nontypable.

The examination of meningococcal strains recovered from patients and healthy carriers not associated with patients (3, 11, 14, 18) revealed marked differences in the serotypes isolated from patients and carriers. Certain serotypes, such as types 2 and 15, were prevalent among isolates from patients (1, 5, 17), whereas other serotypes were rarely isolated from patients yet were common among carriers (1, 3, 5). Thus, only certain serotypes within a serogroup are frequently associated with meningococcal disease.

Most serotypes have distinct outer membrane protein profiles when examined by SDS-PAGE (8, 13). Through the examination of different serotypes and numerous non-serotypable strains, 10 different SDS-PAGE types were found, based on the electrophoretic migration of the one to three major outer membrane proteins. Since all 10 SDS-PAGE types are found among non-serotypable strains, SDS-PAGE typing serves to distinguish among such strains.

There have been several studies on meningococcal carriage within families with and without meningococcal disease (2, 15, 16, 20, 22), but few studies used both serogrouping and serotyping information (6, 7). Therefore, we studied meningococcal strains isolated from families of meningococcal disease patients, characterizing them as to serogroup, serotype, and SDS-PAGE type. In 30% of the families, we found carriage of two or more meningococcal strains. The mother was found most likely to be a carrier of the patient's strain.

MATERIALS AND METHODS

Strains and growth conditions. Strains with CM prefixes, S prefixes, and MM prefixes were obtained from, respectively, Allan Ronald, University of Manitoba, Winnipeg, Canada; Harry Feldman, Upstate Medical Center, Syracuse, N.Y.; and Melvin Marks, Montreal Children's Hospital, Montreal, Canada. Other strains were obtained in the Washington, D.C., area by us. The strains were grown on brain heart infusion medium (Difco Laboratories, Detroit, Mich.) containing 1% normal horse serum and stored as described previously (4). The strains were serogrouped by the antiserum agar technique (4).

STA preparation. A 6- to 8-h growth from brain heart infusion medium containing 1% normal horse serum was inoculated into 200 ml of tryptic soy broth

(Difco Laboratories) and grown overnight at 36°C on a gyratory shaker at 150 rpm. The cells were harvested by centrifugation at 10,000 × *g* for 15 min and then suspended without washing in 5 ml of 0.2 M lithium chloride–0.1 M sodium acetate (pH 6.0). The cells were extracted at 45°C for 2 h with vigorous shaking in the presence of 4-mm glass beads. The cell-free extracts were recovered by centrifugation at 10,000 × *g* for 15 min. The serotype antigen (STA) was pelleted from the extract by centrifugation at 100,000 × *g* for 2 h; it was suspended in water, repelleted, and finally taken up in 0.5 ml of distilled water containing 0.02% (wt/vol) sodium azide.

Serotype identification. The STAs were examined by double diffusion in agar gel (10) with typing sera prepared against the purified prototype STAs (12).

SDS-PAGE typing. The STAs were examined by SDS-PAGE with the Weber-Osborn neutral phosphate buffer system in a slab gel apparatus (11). Briefly, 20-μl samples containing 1 to 2 mg of protein per ml were mixed with 20 μl of 2% (wt/vol) SDS–2% (vol/vol) 2-mercaptoethanol in 8 M urea and heated at 100°C for 2 min. The samples were electrophoresed on gels containing 10% (wt/vol) acrylamide and 0.3% (wt/vol) bisacrylamide. The protein bands were stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.). Photographs were made with Polaroid type 57 film. Strains were typed by comparing the photographs with those of reference SDS-PAGE type patterns and by the use of internal standards consisting of known reference strains.

Statistical method. The data were evaluated for statistical significance by using the chi-square test.

RESULTS

We examined meningococcal isolates from contacts, mostly family members, of 27 meningococcal disease patients. All disease isolates were from unrelated sporadic cases, primarily from the United States and Canada. For a comparison of disease and contact isolates, we considered only those disease isolates for which there was at least one associated contact carrier isolate.

To identify the relatedness of meningococcal strains from patients and their contacts, the meningococcal isolates were characterized by their serogroup, serotype, and SDS-PAGE type. The strains were predominantly serogroups B and C (data not shown). The overall distribution of serotypes and SDS-PAGE types among the 81 meningococcal strains studied is shown in Table 1. Serotype 2 and non-serotypable strains accounted for 26 and 37%, respectively, of the isolates, predominating among both patient and contact carrier isolates. Although 48% of the disease isolates were SDS-PAGE type I, this SDS-PAGE type was isolated from only 26% of the contacts.

Serogroup determination alone does not necessarily provide an accurate representation of the patient-carrier relationship within a family. Further examination of meningococcal isolates by serotyping and SDS-PAGE typing indicated that more than one meningococcal strain of the same serogroup could be present in a single family. Representative results of such analyses are shown in Table 2 (see also Table 5 of reference 8). In 70% of cases, all contact carriers had strains indistinguishable from the disease isolate. However, in the other 30%, members of the same family carried either strains of different serotypes of the same serogroup (e.g., patient 4) or strains of multiple serogroups (patients 16 and 22). SDS-PAGE typing was particularly useful in cases in which the isolates were non-serotypable (patient 14).

TABLE 1. Distribution of serotypes and SDS-PAGE types among 27 patient isolates and 54 contact carrier isolates

Identification method	Type	No. of isolates	
		Patient	Carrier
Serotype	1	3	5
	2	10	11
	6	1	5
	14	2	6
	Other	2	6
	NT[a]	9	21
SDS-PAGE type	I	13	14
	II	6	14
	III	0	4
	IV	6	16
	IX	2	6

[a] NT, Nontypable.

In 6 of 27 families, all isolates were of the same serogroup but were non-serotypable. In these cases, the outer membrane protein patterns of the isolates served to determine whether they were related (Fig. 1). In some cases, the SDS-PAGE patterns of one or more contact strains were different from the SDS-PAGE pattern of the patient's strain (Fig. 1A). The most frequent finding was that patient isolates and contact isolates had identical patterns on SDS-PAGE (Fig. 1B). In these cases, almost all protein bands, major and minor, of the contact isolates corresponded exactly to those of the patient's isolate. Some variability was observed in the lower-molecular-weight major proteins (approximately 28,000 molecular weight) in otherwise identical strains obtained from patients and contacts (Fig. 1C).

The distribution of meningococcal strains among the contacts of 24 children with meningococcal disease was examined with regard to the carrier's relationship to the patient (Table 3). The patients were unrelated epidemiologically. Both parents and all available siblings were cultured, but the total number of siblings was not known. Of the 52 contact carriers, 60% were

TABLE 2. Patient isolates and associated contact carrier isolates from individual families[a]

Patient	Carrier	Age	Specimen	Strain	Serogroup	Serotype	SDS-PAGE type
4		3 mo	CSF	CM-103	B	2	I
	Mother		NP	CM-104	B	2	I
	Sibling 1	7 yr	NP	CM-105	B	6	III
	Sibling 2	5 yr	NP	CM-106	B	6	III
7		1 yr	CSF	S-3446-1	B	14	IX
	Sibling		NP	S-3446-2	B	14	IX
	Playmate		NP	S-3446-3	B	14	IX
14		17 mo	CSF	S-5177	B	NT	II
	Father		NP	S-5179	B	NT	II
	Mother		NP	S-5180	B	NT	II
16		1 yr	CSF	S-5782	C	2	I
	Father		NP	S-5789	Y	14	IX
	Mother		NP	S-5788	C	2	I
22		1 yr	CSF	MM-39	C	2	I
	Father		NP	MM-40	NG	NT	II
	Mother		NP	MM-2	B	NT	II
	Sibling	8 yr	NP	MM-1	C	2	I

[a] CSF, Cerebrospinal fluid; NP, nasopharynx; NT, non-serotypable; NG, nongroupable.

adults. Among household contacts, the mother was most likely and the father was least likely to be carrying the disease isolate. In 8 of the 24 cases, the mother was the only carrier, whereas the father was the only carrier in 1 of 24 cases. This difference was statistically significant at $P < 0.05$. Nonhousehold contacts were least likely to be carrying the disease isolate.

DISCUSSION

Our studies on household meningococcal carriage made use of both serotyping and SDS-PAGE typing procedures for more precise strain identification. The SDS-PAGE typing procedure can be a useful tool for identifying epidemiologically related strains. Whereas serotyping reagents are available in relatively few laboratories, any laboratory having experience wih polyacrylamide gel electrophoresis can perform SDS-PAGE typing.

Most studies on meningococcal carriage within households (2, 15, 20) were only able to show that the individuals carried meningococcal strains of the same serogroup and, in some cases, of the same sulfonamide sensitivity. Our studies show that patient isolates and contact carrier isolates were different in approximately 30% of families. That more than one serogroup may be present in a family was first shown by Silverthorne (22) in studies of 51 sporadic cases occurring over a 6-year period. He found that in 36% of case contact families, one or more carriers had strains of a serogroup different from that of the patient.

We found that 60% of carriers were adults, which agrees with the findings of Norton and Baisley (20), who studied the familial contacts of 1,272 patients during the 1928 to 1929 meningococcal disease outbreak in Detroit, Mich. They found that 54% of carriers were adults. Although both parents were likely to be meningococcal carriers, we found that the father was less likely to carry the patient's strain. However, in studies during a group B meningococcal disease outbreak, Foster et al. (7) found higher levels of carriage among children than adults (57 versus 20%). They also observed that high carriage rates with the group B disease strain occurred only among intimate household contacts.

We found that the mother was most likely to carry the disease isolate among the familial contacts of children with meningococcal disease. These results are in agreement with carrier studies in Bolten, England (6). When only families with two or more children were considered, 77% of the children with meningococcal disease were the youngest family member. However, Munford et al. (19) found that the distribution of familial carriers was different when the patient was an adult. They found an inverse relationship between the age of the patient and carriage rates in the household.

High carriage rates generally occur in the families of meningococcal disease patients. In studies of sporadic cases by Farries et al. (6) and Olcén et al. (P. Olcén, J. Kiellander, D. Danielsson, and E. I. Linquist, Scand. J. Infect. Dis., in press), 34 and 41% carriage rates, respectively, were found within the families of meningococcal

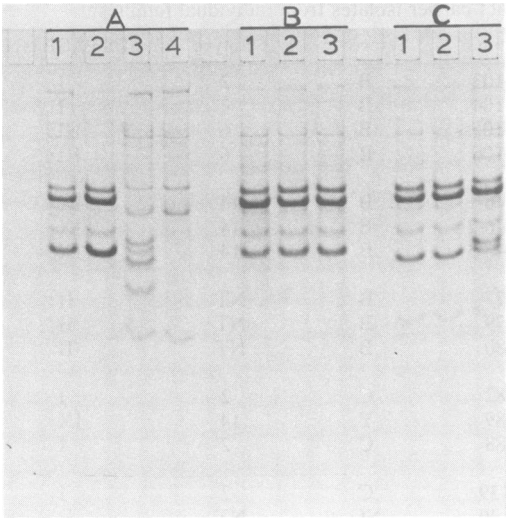

FIG. 1. Examination of meningococcal strains recovered from the families of three patients, A, B, and C, by SDS-PAGE analysis of outer membrane fractions. (A) Lane 1, spinal fluid isolate from patient; lane 2, throat isolate from sibling 1; lane 3, throat isolate from sibling 2; lane 4, throat isolate from father. (B) Lane 1, blood isolate from patient; lane 2, throat isolate from father; lane 3, throat isolate from mother. (C) Lane 1, spinal fluid isolate from patient; lane 2, throat isolate from sibling 1; lane 3, throat isolate from sibling 2.

disease patients. However, when carriers were present in disease-free families, carriage rates within these families were quite similar to those in families with meningococcal disease (18, 20). A recent study by Marks et al. (18) showed no differences in carriage rates between the contacts of patients and the contacts of healthy carriers. There was, however, a marked difference in the SDS-PAGE types in that 34% of the contacts of patients carried SDS-PAGE type I organisms, whereas only 9% of the contacts of healthy carriers had SDS-PAGE type I strains.

TABLE 3. Frequency of disease isolate carriage among contacts of 24 epidemiologically unrelated children with meningococcal disease[a]

Contact(s)	No. of carriers	Carriers with disease isolate	
		No.	%
Mother	16	13	81
Father	12	6	50
Sibling(s)	19	14	74
Other	5	2	40

[a] It was assumed that all families had a mother and a father. All available family members were cultured for possible meningococcal carriage, but the total number of siblings in each family was unknown.

Whether meningococcal disease occurs within a family is determined not only by the immunological susceptibility of the family members but also by the relative virulence of the meningococcal strain. In two recent studies, over 60% of group B and group C cases were caused by serotype 2 (1, 5), yet this serotype was found in ≤2% of carriers when case contacts were eliminated (1, 3). In contrast, other group B serotypes, such as 4, 6, and 14, have almost never been isolated from patients but are common among healthy carriers (1, 5, 11).

Only patients who had at least one meningococcal carrier in their family were included in this study. An indication of the number of cases in which no familial carriers were found is obtained from the studies of Olcén et al. (Olcén et al., in press). They were unable to isolate meningococci from any family member in 5 of 21 (24%) consecutive meningococcal disease cases. In a study by Sippel and Girgis (23), pharyngeal carriage by the patient was related to carriage rates found in the patient's family. Sippel and Girgis found that 40% of familial contacts were carriers of the same serogroup as the patient when the patient had the organism in his throat at the time of hospital admission; they found a 23% carriage rate among household contacts when the patient did not harbor the organism in his throat at the time of hospital admission.

Greenfield et al. observed that the duration of carriage of the same serogroup was 10 months or more (16), whereas Rake found that 4 of 10 laboratory personnel remained carriers for over 20 months (21). It is difficult to interpret these observations since studies of long-term carriage among workers in our laboratory (unpublished data) indicate that some individuals may remain carriers of the same serogroup yet lose one strain and acquire another with a distinctly different SDS-PAGE type.

Epidemiological studies of meningococcal disease have traditionally relied on serogroup identification and sulfonamide sensitivity for strain identification. Strain identification for epidemiological purposes must also include serotyping, SDS-PAGE typing, or both techniques. The latter methods are applicable primarily to the pathogenic B, C, Y, and W135 serogroups, since strains of group A, the other pathogenic serogroup, are not readily distinguishable by serotyping methods which rely on the immunological specificity of the major outer membrane proteins. Group A strains may, however, be further differentiated by their lipopolysaccharide types (24). Our results indicate that multiple strains may be carried within a household, but they do not provide evidence concerning who within the household initiates the infection. Studies within defined populations may provide more informa-

tion concerning transmission and carriage, provided serotyping and SDS-PAGE typing techniques are employed.

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