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Variability of Low-Molecular-Weight, Heat-Modifiable Outer Membrane Proteins of *Neisseria meningitidis*

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Analysis of major outer membrane protein (MOMP) profiles of various meningococci by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of 0 to 2 low-molecular-weight, heat-modifiable MOMPs (molecular weight, 25,000 to 32,000) and 1 to 3 high-molecular-weight MOMPs (molecular weight, 32,000 to 46,000). Heat modifiability was investigated by comparing MOMP profiles after heating in SDS solutions at 100°C for 5 min or at 40°C for 1 h. Low-molecular-weight MOMPs shifted to higher apparent molecular weights after being heated at 100°C. Heat modifiability of high-molecular-weight MOMPs varied among strains; whenever modified these proteins shifted to lower apparent molecular weights after complete denaturation. Variability of low-molecular-weight, heat-modifiable MOMPs was demonstrated when MOMP profiles were compared of (i) isolates from index cases and associated cases and carriers among contacts, (ii) different isolates from the same individual, and (iii) isolates from a small epidemic caused by serogroup W-135. In some cases high-molecular-weight MOMPs revealed quantitative differences among related strains. The observed variability and quantitative differences indicate that MOMP serotyping and typing on the basis of SDS-PAGE profiles (PAGE typing) need careful reevaluation.

Meningococci can be treated with various combinations of salts (NaCl, LiCl, $CaCl_2$, sodium acetate) with or without ethylenediaminetetraacetic acid (6, 8, 17, 26, 27) to extract so-called outer membrane complexes (OMC) or serotype antigen complexes. OMC (or serotype antigen complexes) consist of, on the one hand, group-specific capsular polysaccharides and, on the other hand, lipopolysaccharides and outer membrane proteins, both containing type-specific immunodeterminants (6, 17, 27). A few predominant proteins can be identified after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE): the so-called major outer membrane proteins (MOMPs) (7, 18).

Meningococcal MOMPs can be divided into two classes on the basis of heat modifiability and solubility in detergents (8). When analyzed by SDS-PAGE, high-molecular-weight MOMPs shift to lower apparent molecular weights after complete denaturation in SDS solutions. High-molecular-weight MOMPs are barely soluble in detergents less agressive than SDS, such as Triton X-100 and deoxycholate. Low-molecular-weight MOMPs shift to higher apparent molecular weights after complete denaturation in SDS solutions and are soluble in Triton X-100 and deoxycholate.

Meningococcal MOMPs are used for serotyping and PAGE typing (typing based on SDS-PAGE profiles) because they show a wide range of strain-specific variations (5, 7, 18, 27). Some of the MOMP serotype antigens are associated with virulence, especially the serotype *2a*, *2b*, and *2c* antigens (5, 18). Since a restricted number of serotypes is predominant in patients, these serotype antigens are used for serodiagnostic and immune prophylactic investigations (4, 5, 23, 28).

It has been demonstrated that the low-molecular-weight, heat-modifiable MOMPs of the related organism *Neisseria gonorrhoeae* are highly variable (13, 24, 25). To investigate the variability of meningococcal MOMPs, we have compared MOMP profiles of (i) isolates from index cases and associated cases and carriers among contacts, (ii) isolates from the same individual, and (iii) isolates from an epidemic caused by serogroup W-135 in The Netherlands.

MATERIALS AND METHODS

Bacterial strains and cultural conditions. The meningococci used in this study were taken from our collection, which consists of meningococcal strains sent to our laboratory since 1959 by the hospital and public health laboratories in The Netherlands. Three categories of isolates were selected: (i) from index cases and associated cases and carriers among con-

tacts, (ii) from different sites or different periods from one host, and (iii) from a small epidemic caused by serogroup W-135. From the various transport media the strains were passaged two to six times in vitro to Mueller-Hinton agar plates (Difco) supplemented with yeast extract. The strains were stored by lyophilization and at −70°C in glycerol-peptone (8% [wt/vol] glycerol-1% [wt/vol] proteose peptone no. 3 [Difco]). Strain 3006 represents one of the prototype serotype *2b* strains (18).

Sulfadiazine sensitivity. Sulfadiazine sensitivity of meningococci was determined by the methods described by Abbott et al. (1). The dilution series used was: 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 10, 50, and 100 μg/ml.

Serogrouping and serotyping. Meningococci were serogrouped by agglutination and microprecipitation (in agar gel) and serotyped by a modified microprecipitation procedure as described previously (18). The serotypes involved were supplemented with serotypes 13, 14, and 15. Prototype strains BC-4 (group B, serotype 13), S-3446-1 (group B, serotype 14), and H-355 (group B, serotype 15) were obtained from C. E. Frasch and were used for immunizing rabbits (18). The antisera were absorbed to make them specific for microprecipitation.

Isolation and characterization of meningococcal OMC. OMC were isolated from meningococci grown overnight in tryptic soy broth cultures (Difco) with the LiCl-ethylenediaminetetraacetic acid method as described previously (17). OMC were analyzed by means of SDS-PAGE as described by Laemmli (12). OMC were heated in 2% (wt/vol) SDS, 10% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, and 0.062 M tris(hydroxymethyl)aminomethane hydrochloride (pH 6.8) at 100°C for 5 min or at 40°C for 1 h. The following proteins (Boehringer) were used as molecular weight markers: cytochrome *c* (molecular weight, 12,500), chymotrypsinogen A (molecular weight, 25,000), aldolase (subunit molecular weight, 39,500), ovalbumin (molecular weight, 45,000), catalase (subunit molecular weight, 60,000), bovine albumin (molecular weight, 67,000). Proteins were stained by Coomassie brilliant blue (17).

RESULTS

Heat modifiability and molecular weight limits of meningococcal MOMPs. The heat modifiability of a number of strains was detected by means of comparison of MOMP patterns of outer membrane complexes prepared for SDS-PAGE at 100°C for 5 min and at 40°C for 1 h. Strains 750352 I (serogroup A), 3624 (serogroup B, serotype *2a*), 2861 (serogroup C, serotype *2a*) (these strains are described in Tables 1 and 2), and 3006 (serogroup B, prototype *2b*) were compared by means of these methods (Fig. 1).

It appeared that the heat modifiability of high-molecular-weight MOMPs varied among strains and individual MOMPs under the experimental conditions (arrows indicate the predominant MOMPs involved).

Some high-molecular-weight MOMPs migrated into very high molecular weight bands (molecular weight, ca. 120,000) at the lower temperature. In all cases low-molecular-weight MOMPs shifted to higher apparent molecular weights after complete denaturation (molecular weight, 25,000 to 32,000). The border between increased or decreased apparent MOMP molecular weights as a result of heat modification thus seems to lie at ca. 32,000.

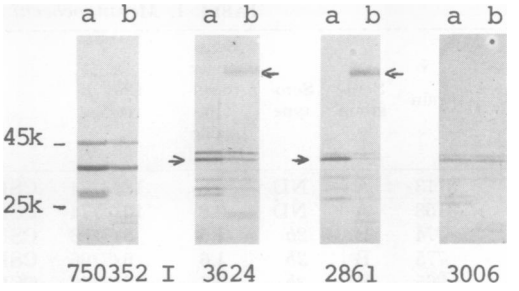

FIG. 1. *Heat modifiability of meningococcal MOMPs was tested by comparing MOMP profiles after heating in SDS at 100°C for 5 min or at 40°C for 1 h (a = 100°C; b = 40°C). The following strains were tested: 750352 I (serogroup A, Table 2), 3624 (serogroup B, Table 2), 2861 (serogroup C, Table 1), and 3006 (serogroup B, prototype 2b).*

Isolates from index cases and associated cases and carriers among contacts. The meningococcal strains isolated from index cases and associated contacts used in this study are listed in Table 1. The MOMP patterns of these strains are depicted in Fig. 2. Variations were found not only among independently isolated strains, as we expected, but were also observed among related strains. Two serogroup A strains were examined (3113 and 3153) which were isolated 3 weeks apart from a married couple. The strains displayed the same sensitivity to sulfadiazine, but were different with respect to the MOMPs of molecular weights 27,000 and 45,000 (Fig. 2). It should be noted that serogroup A strains have not been serotyped because the existing MOMP serotypes do not occur in group A strains (18).

All serogroup B isolates studied except one (2384) were classified as serotype *2b* and contained the MOMPs characteristic for serotype *2b* strains (18) with molecular weights of 41,500 and 42,500 (Fig. 2). Differences with respect to low-molecular-weight MOMPs were observed between the following related strains: 2218 and 2221; 2496 and 2497; 2991 and 2992; 3531 and 3581. Strains 3531 and 3581 were isolated within 1 month of each other, and the others were isolated within a few days. Identical MOMP patterns were found between other related strains: 774 and 775; 865 and 875; 1090 and 1093.

TABLE 1. *Meningococcal strains isolated from contacts*[a]

Strain	Sero-group	Sero-type	Sensitivity to sulfadiazine (μg/ml)	Date of receipt	Source	Age (yr)	Sex	Relationship
3113	A	ND	0.8	18/3/74	CSF	?	M	Married couple
3153	A	ND	0.8	10/4/74	CSF	52	F	
774	B	*2b*	1.6	5/7/66	CSF	1	F	Sister and brother
775	B	*2b*	1.6	5/7/66	CSF	3	M	
865	B	*2b*	0.4	6/12/66	CSF	3	F	Sisters
875	B	*2b*	0.8	13/12/66	CSF	4	F	
1090	B	*2b*	0.8	22/8/67	CSF	3	M	Brother and sister
1093	B	*2b*	0.8	30/8/67	Blood	?	F	
2218	B	*2b*	0.4	7/12/71	CSF	1	F	Daughter and mother
2221	B	*2b*	0.4	10/12/71	Nasopharynx	53		
2384	B	NT	0.2	19/4/72	CSF	?	?	Household
2398	B	*2b*	0.8	4/5/72	CSF	1	F	
2496	B	*2b*	1.6	24/8/72	CSF	2	M	Brother and sister
2497	B	*2b*	1.6	28/8/72	Blood	3	F	
~~2991~~	B	*2b*	0.8	7/1/74	CSF	11/12	M	Brothers
2992	B	*2b*	0.8	7/1/74	CSF	2	M	
3531	B	*2b*	0.8	11/3/75	CSF	1	F	Sister and brother
3581	B	*2b*	0.8	15/4/75	CSF	4	M	

[a] ND, Not determined; NT, not typable.

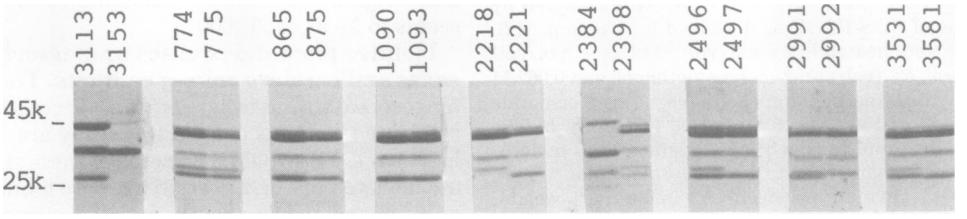

FIG. 2. *MOMP profiles of meningococci isolated from contacts. Detailed characteristics of the strains involved are given in Table 1.*

Strains 2384 (nontypable) and 2398 (serotype *2b*) isolated 3 weeks apart within one family showed completely different MOMP patterns, and sulfadiazine sensitivity differed by two dilution steps. These findings indicate that there is no relation between strains 2384 and 2398. The sulfadiazine sensitivity within each of the other related pairs was either equal or differed by only one dilution step. Randomly selected strains revealed a wide range of sulfadiazine sensitivities. No relationship between sulfadiazine sensitivity and the occurrence of certain MOMP patterns could be recognized (Fig. 2; Table 1).

Different isolates from one host. The meningococcal strains isolated from one host are listed in Table 2. MOMP patterns of these strains are shown in Fig. 3.

Among the serogroup A strains investigated, strains 750352 I and II (blood and cerebrospinal fluid [CSF] isolates) showed different low-molecular-weight MOMP patterns. Within the strain pairs 760115 and 760127 and 760133 and 760134, the same MOMP patterns were revealed. All strains exhibited mutually identical sulfadiazine sensitivities. From Fig. 2 and 3 we concluded that the serogroup A strains studied all contained an MOMP with a molecular weight of 36,000.

Among the serogroup B strains studied, strains 3217, 3218, and 3230 belonged to serotype *2b*, displayed the same sensitivity to sulfadiazine (one dilution step difference is regarded to be identical), and contained the same MOMP patterns; strain 3218 (isolated from the nasopharynx) revealed one extra low-molecular-weight MOMP (Fig. 3). Strains 3349 and 3624 were isolated half a year apart from the nasopharynx of the same host; these strains were different with respect to their serotype (serotypes 1 and *2a*, respectively), sulfadiazine sensitivity, and MOMP patterns (all of which indicate that these

TABLE 2. *Meningococcal strains isolated from one individual*[a]

Strain	Sero-group	Serotype	Sensitivity to sulfadiazine (μg/ml)	Date of receipt	Source	Age (yr)	Sex
750352 I	A	ND	0.4	4/12/75	Blood	16	M
750352 II	A	ND	0.4	4/12/75	CSF	16	M
760115	A	ND	0.4	25/2/76	Blood	28	M
760127	A	ND	0.4	26/2/76	CSF	28	M
760133	A	ND	10	3/3/76	Blood	28	M
760134	A	ND	10	3/3/76	CSF	28	M
3217	B	*2b*	1.6	31/5/74	CSF	19	F
3218	B	*2b*	1.6	31/5/74	Nasopharynx	19	F
3230	B	*2b*	3.2	31/5/74	Blood	19	F
3349	B	1	0.2	8/11/74	Nasopharynx	26	F
3624	B	*2a*	0.8	15/5/75	Nasopharynx	26	F
790666 I	B	NT	0.4	1/8/79	Blood	26	F
790666 II	B	NT	0.4	1/8/79	Skin	26	F
790666 III	B	NT	0.4	1/8/79	Brains	26	F
790666 IV	B	NT	0.4	1/8/79	Heart	26	F
790666 V	B	NT	0.4	1/8/79	Abdomen	26	F
1942	C	13	0.4	16/12/70	Blood	2/12	M
1943	C	13	0.4	16/12/70	CSF	2/12	M
1947	C	13	0.4	16/12/70	Nasopharynx	2/12	M
2874	C	15	0.8	22/8/73	CSF	8	F
2875	C	15	0.4	22/8/73	Blood	8	F
2876	C	15	0.4	22/8/73	Nasopharynx	8	F
3159	C	13	6.4	18/4/74	Nasopharynx	23	M
3169	C	13	6.4	26/4/74	Nasopharynx	23	M
3185	C	13	6.4	3/5/74	Nasopharynx	23	M
3197	C	13	10	14/5/74	Nasopharynx	23	M

[a] ND, Not determined; NT, not typable.

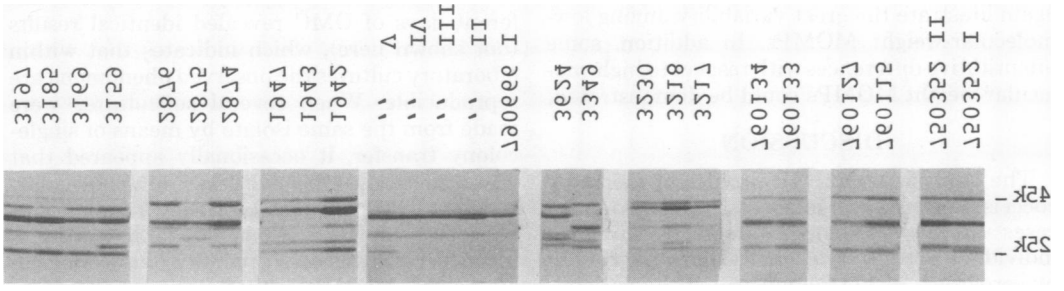

FIG. 3. *MOMP profiles of meningococci isolated from one host. Detailed characteristics of the strains involved are given in Table 2.*

strains were different). Strains 790666 I through V (isolated from blood, skin, brain, heart, and abdomen) exhibited the same sulfadiazine sensitivity and were nontypable, and all showed the same MOMP patterns with the exception of strain 790666 (abdominal isolate, obtained at necropsy), which contained an extra, low-molecular-weight MOMP.

Among the serogroup C isolates investigated, strains 1942, 1943, and 1947 (isolated from blood,

CSF, and nasopharynx) were identical with respect to their serotype (serotype 13), sulfadiazine sensitivity, and MOMP patterns (Fig. 3; Table 2). Although the amount of protein loaded on the gel is not the same in all cases, it is evident that the same MOMPs are present in similar ratios. Strains 2874, 2875, and 2876 (isolated from blood, CSF, and nasopharynx) contained the same serotype antigen (serotype 15) and exhibited the same sulfadiazine sensitivity. However, strain 2875 (isolated from blood) contained a different low-molecular-weight MOMP and fewer high-molecular-weight MOMPs as compared to strains 2874 and 2876. Strains 3159, 3169, 3185, and 3197 (consecutive nasopharynx isolates) were identical as to their serotype (serotype 13) and sulfadiazine sensitivity. Strain 3159 revealed one extra low-molecular-weight MOMP, and strain 3185 contained fewer high-molecular-weight MOMPs. It can be concluded from Fig. 3 that no particular MOMP profiles could be associated with the site of isolation. Low-molecular-weight MOMPs demonstrated variability and high-molecular-weight MOMPs may differ quantitatively among related strains.

Serogroup W-135 isolates. Serogroup W-135 was not recognized till 1971 in The Netherlands; since then an increasing number of serogroup W-135 strains were isolated both from cases and carriers, reaching a maximum of 20 cases a year both in 1974 and 1975. About 90% of serogroup W-135 cases (18) belonged to serotype *2a*. These figures indicate that serogroup W-135 strains isolated from cases in The Netherlands represent a small epidemic. Eight randomly selected serogroup W-135, serotype *2a*, strains from cases revealed nearly identical sulfadiazine sensitivity and high-molecular-weight MOMP patterns (Fig. 4). Sulfadiazine sensitivity varied between 0.2 and 1.6 μg/ml. These strains again illustrate the great variability among low-molecular-weight MOMPs. In addition, some quantitative differences with respect to high-molecular-weight MOMPs could be demonstrated.

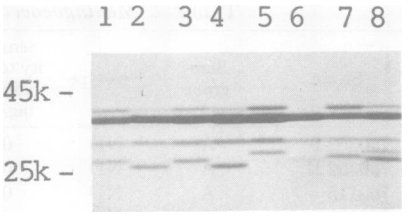

FIG. 4. *MOMP profiles of serogroup W-135, serotype 2a, meningococci isolated from a small epidemic in The Netherlands.*

Lane no.	Strain	Sensitivity to sulfadiazine (μg/ml)	Date of receipt	Source	Age (yr)	Sex
1	750020	0.8	9/7/75	Blood	12	M
2	750027	0.2	10/7/75	CSF	14	M
3	750040	0.8	18/7/75	CSF	30	F
4	750058	0.4	30/7/75	Blood	7/12	M
5	760037	0.8	15/1/76	Blood	?	M
6	760269	0.8	22/4/76	CSF	15	?
7	760579	1.6	1/9/76	Blood	8/12	F
8	760728	0.8	23/11/76	CSF	1	M

DISCUSSION

The analysis of MOMP profiles of meningococci isolated from (i) index cases and associated cases and carriers among contacts, (ii) the same individual, and (iii) a small epidemic caused by serogroup W-135 demonstrated the variability of low-molecular-weight, heat-modifiable MOMPs. One possible explanation for this variability might be that different human hosts, on the one hand, and different sites or different times of isolation from a single host, on the other hand, reflect different physiological conditions which select meningococci with different variable, low-molecular-weight MOMPs. Such a process resembles the one associated with gonococcal low-molecular-weight MOMPs in which gonococci express depending upon the physiological conditions of the host (10). Another explanation could be laboratory selection.

Gonococci with different low-molecular-weight MOMPs and pili can be differentiated within clinical isolates by means of colony morphology (13, 14, 20, 24). However, meningococci have not shown a clear-cut relationship between colony morphology and the presence of pili, nor will they probably with low-molecular-weight MOMPs (2, 9, 15, 16). To minimize laboratory selection, we routinely passage our strains nonselectively. Various duplicate isolations on different days of OMC revealed identical results (not shown here), which indicates that within laboratory cultures the observed phenomena are reproducible. When several subcultures were made from the same isolate by means of single-colony transfer, it occasionally appeared that the extracted OMC had different low-molecular-weight MOMP profiles (not shown here). This illustrates the heterogeneous character of meningococcal cultures. Variability of low-molecular-weight MOMPs within various laboratory subcultures from one isolate emerged less frequently than between the related strains studied. Probably selection by the physiological condition of the host and laboratory treatments both contributed to the observed variability of low-molecular-weight MOMPs.

Whatever the true explanation for the observed variability may be, this quality must be

regarded as an intrinsic property of meningococci supplying the meningococcus a means for adaptation to varying external conditions. Recent investigations (3, 11, 21) revealed the presence of metastable genes in *Escherichia coli* and *Salmonella typhimurium* which control the expression of pili and flagella. Whether this is also the case for *Neisseria gonorrhoeae* and *Neisseria meningitidis* with respect to low-molecular-weight MOMPs and pili remains to be examined.

Meningococci appeared to contain 0 to 2 low-molecular-weight, heat-modifiable MOMPs with molecular weights of 25,000 to 32,000, showing a close biochemical relationship by means of their heat modifiability and variability. In addition, one to three MOMPs with molecular weights of 32,000 to 46,000 can be demonstrated within the meningococcal outer membrane, showing considerable biochemical differences by means of their heat modifiability and quantitative expression (copies per cell). Further characterization of these proteins is necessary.

Although serogroup A strains appeared to be more homogeneous with respect to MOMP profiles than serogroup B and C strains (all group A strains contain a MOMP molecular weight of 36,000), differences could be observed among group A strains both in high- and low-molecular-weight MOMPs, in contradiction to other investigations (22). Group A low-molecular-weight MOMPs appeared to possess strain-specific immunodeterminants (29). The data presented here indicate that low-molecular-weight MOMPs are not suitable for typing purposes because of their great variability. However, current serotyping schemes do involve these proteins (7, 18, 29), which indicates the need for reevaluation of such schemes. MOMP typing should be restricted to high-molecular-weight MOMPs, although quantitative differences in these proteins have been observed among related strains, probably due to varying in vitro expression (7). This remains to be examined. In addition to serotyping and PAGE typing, detection of sulfadiazine sensitivity could be used for differentiation to some extent.

It is interesting to note that low-molecular-weight meningococcal MOMPs seem to induce strong antibody responses in human beings after meningococcal diseases as detected by the gel immunoradioassay (19). Immunogenicity and variability of low-molecular-weight MOMPs might reflect a mechanism of antigenic drift. The antibody responses of the human host will be extended in a later paper.

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