JOURNAL OF CLINICAL MICROBIOLOGY, July 1991, p. 1486–1492
0095-1137/91/071486-07$02.00/0

Vol. 29, No. 7

Characterization of Serogroup A and B Strains of *Neisseria meningitidis* with Serotype 4 and 21 Monoclonal Antibodies and by Multilocus Enzyme Electrophoresis

E. WEDEGE,[1]* D. A. CAUGANT,[1] L. O. FRØHOLM,[1] AND W. D. ZOLLINGER[2]

Department of Methodology, National Institute of Public Health, 0462 Oslo 4, Norway,[1] and Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307[2]

Received 28 January 1991/Accepted 25 April 1991

The reactions of serogroup A strains of *Neisseria meningitidis* with one monoclonal antibody specific for serotype 21 and three different monoclonal antibodies specific for serotype 4 were compared with those of serogroup B strains previously assigned to serotype 4. Antibody binding was studied by enzyme-linked immunosorbent assay (ELISA), dot blotting, and immunoblotting. Characterization of the isolates by the electrophoretic mobilities of 14 metabolic enzymes showed 50 multilocus enzyme genotypes. All except two genotypes fell into three distinct clusters: I, IIa, and IIb. The enzyme genotypes of serogroup B strains were mainly in cluster I, and 88% of the serogroup A strains had genotypes in clusters IIa and IIb. Serogroup B strains generally reacted with all three serotype 4 monoclonal antibodies in ELISA and dot blotting but with only two in immunoblots. Serogroup A strains showed two different reactions in the blotting methods: either binding of the serotype 21 antibody only or binding of this and two of the three serotype 4 monoclonal antibodies. Strains of the first pattern were in clusters I and IIa, whereas all but two strains in cluster IIb were of the second pattern. In ELISA, an additional reaction of two of the serotype 4 monoclonal antibodies with serogroup A isolates was observed. The different binding of these two monoclonal antibodies in ELISA and the blotting methods appeared to result from heat inactivation of the meningococcal cells and use of detergent-containing reagents in ELISA. The results show that the serotype of serogroup A strains is distinct from serotype 4 of serogroup B strains.

The serotype specificity of *Neisseria meningitidis* is based on antigenic differences in the outer membrane porin proteins, which are either class 2 or 3 proteins according to the molecular weight (9). Until now, 20 different serotypes have been proposed, and monoclonal antibodies (MAbs) have been made against the majority of these (3, 9, 17, 28).

The class 3 proteins of serogroup A strains were found to be antigenically homogeneous and of similar molecular weights (14, 18, 21, 27) and were designated serotype 21 (9). Recently, a new classification of serogroup A strains was described on the basis of the electrophoretic mobilities of cytoplasmic enzymes and of the class 1 and 6 outer membrane proteins (15). This combined analysis differentiated four clonal groups among serogroup A strains. Strains in each clonal group had distinct class 1 protein subtypes, and in enzyme-linked immunosorbent assay (ELISA), they bound serotype-4-specific MAbs (6). Because both the serotype 21 (ATCC 13077) and the serotype 4 (M981) reference strains reacted with these MAbs, it was suggested that serogroup A strains should be reassigned to serotype 4, that is, to the same serotype as some serogroup B isolates (1a, 16).

To examine whether meningococcal serogroup A strains were serotype 4 or 21, the reactions of such isolates with one serotype-21- and three different serotype-4-specific MAbs (serotype 21 and serotype 4 MAbs, respectively) were compared with those of serogroup B strains previously assigned to serotype 4. Antibody binding was analyzed by ELISA, dot blotting, and immunoblotting. The multilocus enzyme genotypes of the isolates were determined by electrophoresis of 14 cytoplasmic enzymes to study the relationship between the MAb binding pattern and the genotype on the basis of a representative sample of structural genes. The serogroup A strains expressed two distinct binding patterns of the serotype-specific MAbs which were related to the genotypes. Another reaction pattern was presented by the serotype 4 serogroup B strains. Our results showed that the four MAbs had different specificities and that the reported serotype 4 classification of serogroup A strains (1a, 16) was caused by an immunoassay-dependent reaction of one of the serotype 4 reagents.

(Parts of this study were presented at the 10th Scottish Scandinavian Conference on Infectious Diseases, Oslo, Norway, May 1989, and at the Seventh International Pathogenic Neisseria Conference, Berlin, Germany, September 1990.)

MATERIALS AND METHODS

Strains. The 73 meningococcal strains studied were as follows: (i) 42 serogroup A isolates, including 21 reference strains for clonal analysis (15) provided by M. Achtman, and (ii) 31 serogroup B isolates previously assigned to serotype 4 in various laboratories by using either whole-cell ELISA or dot blotting methods. The reference strains for serotype 4 (M981) and serotype 21 (ATCC 13077) (9) were included in the collection. The isolates were recovered from patients (68 strains) and healthy carriers (5 strains) in 25 countries in Europe, the Americas, Asia, Africa, and Australia. The collection period was from 1937 to 1988.

Enzyme electrophoresis. Methods for starch gel electrophoresis and selective enzyme staining have been described by Selander et al. (20). The 14 enzymes assayed were malic enzyme, glucose 6-phosphate dehydrogenase, peptidase,

* Corresponding author.

isocitrate dehydrogenase, aconitase, NADP-linked glutamate dehydrogenase, NAD-linked glutamate dehydrogenase, alcohol dehydrogenase, fumarase, alkaline phosphatase, two indophenol oxidases, adenylate kinase, and an unknown dehydrogenase. Each isolate was characterized by its combination of alleles at the 14 enzyme loci, and distinctive multilocus genotypes were designated as electrophoretic types (ETs).

Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred (mismatches), and the clustering was performed from a matrix of genetic distances by the average-linkage method (22). ETs were numbered sequentially according to their positions in the dendrogram (see Fig. 1). With the exception of ET-5, the ET numbers did not correspond with those previously assigned (5).

Serotyping. The isolates were serotyped by dot blotting (26) and ELISA (2), slightly modified as described previously (26). The antigens were heat-inactivated (56°C for 30 min) meningococcal whole-cell suspensions (2) with an optical density of about 0.1 at 650 nm. The suspensions were stored at 4°C. Binding of the MAbs to the antigens in dot blotting and ELISA were detected by peroxidase- and alkaline phosphatase-conjugated anti-mouse immunoglobulins, respectively, obtained from DAKOPATTS a/s and Sigma Chemical Co., St. Louis, Mo.

In some ELISA experiments designed to study the effects of certain assay conditions on the apparent specificities of the MAbs, a different set of reagents (reagent set 2) was used. The plates were incubated for 30 min at 37°C with a blocking buffer containing 0.5% bovine serum albumin, 0.5% casein, 0.2% sodium azide, and 0.01% phenol red dissolved in Dulbecco's phosphate-buffered saline. This buffer was also used to dilute the primary and secondary antibodies, each of which was incubated for 2 h. The plates were washed with Dulbecco's phosphate-buffered saline, and alkaline phosphatase-conjugated anti-mouse immunoglobulins (Kirkegaard and Perry, Inc., Gaithersburg, Md.) were used as the secondary antibody.

Serogrouping. Strains were serogrouped on dot blots by using whole-cell suspensions and MAbs against serogroups A and B (19).

SDS-gel electrophoresis and immunoblotting. Two volumes of whole-cell suspensions were boiled for 5 min in one volume of sample buffer containing 2-mercaptoethanol (12) or subjected to mild dissociating treatment at 45°C for 30 min in 0.5% sodium dodecyl sulfate (SDS) and 0.5% Triton X-100 (1a). Pyronin B was added as tracking dye. Samples of 15 μl were transferred to wells formed by a 15-tooth comb and separated in 12% acrylamide gels (7 by 8 cm; thickness, 0.75 mm) at 200 V (12). The gels were stained in Coomassie brilliant blue or blotted to nitrocellulose filters (23) at 100 V for 1 h. To renature the blotted antigens (24), the blots were incubated overnight with MAbs diluted in 3% bovine serum albumin in phosphate-buffered saline containing 0.25% of the detergent Empigen BB (Albright and Wilson, Cumbria, United Kingdom). Antibody binding was detected by peroxidase-conjugated anti-mouse immunoglobulins (25).

MAbs. Serotype 4 MAbs were developed in the laboratories of C. E. Frasch (2303 C5), J. T. Poolman (MN14G21.17), and W. D. Zollinger (15-1-P4). They will be referred to as 4F, 4P, and 4Z MAbs, respectively. The serotype 21 MAb (14-1-P21) was from W. D. Zollinger, as were those against serogroups A (14-1-A) and B (2-1-B).

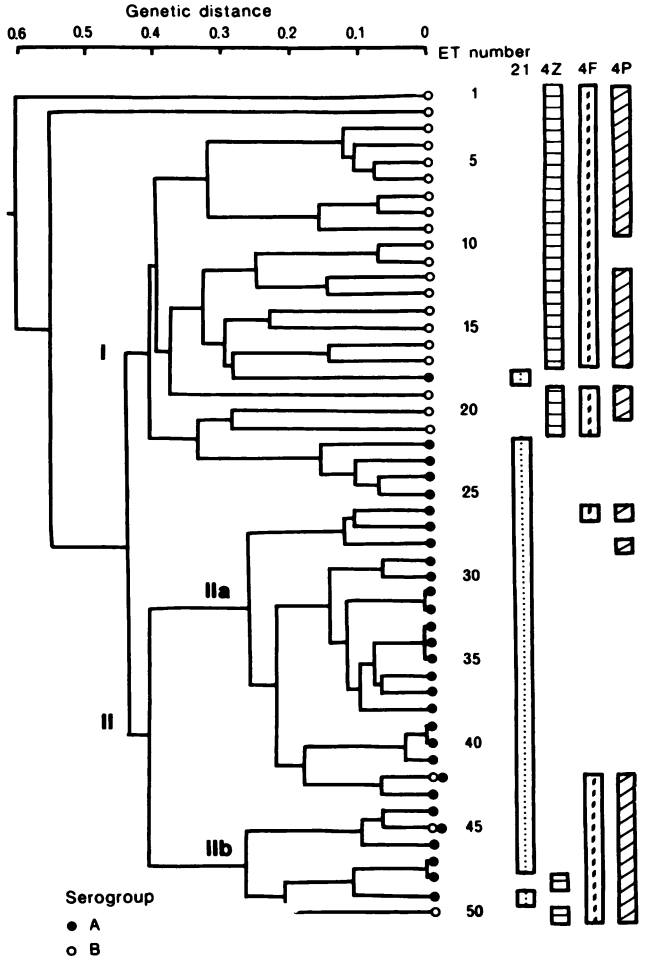

FIG. 1. Genetic relationships among 50 ETs of 42 serogroup A and 31 serotype 4 serogroup B isolates of *N. meningitidis*. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distance between pairs of ETs based on 14 enzyme loci. ETs are numbered sequentially from top to bottom. Reactions on dot blots of the strains with the 21, 4Z, 4F, and 4P MAbs, including both weak and strong bindings, are shown to the right. Lineages containing serogroup A and B strains are marked with filled and open circles, respectively.

RESULTS

Genotype analysis. All 14 enzyme loci were polymorphic, and the number of alleles per locus was 4.4. The 73 strains were assigned to 50 distinct ETs. The genetic relationships among the ETs are shown in Fig. 1. The dendrogram consisted of two distinct clusters of ETs (I and II) and two single ETs (ET-1 and ET-2, each represented by a serogroup B strain) which had no close relationship to the other ETs. The serotype 4 prototype (M981) was ET-2, whereas the serotype 21 prototype (ATCC 13077) was ET-46.

The 23 ETs in cluster I included the genotypes of 31 isolates, of which 26 were serogroup B. ETs 3 through 6 represented clones of the ET-5 complex (4), which has been causing outbreaks and epidemics in various parts of the world. The eight serogroup B strains of the ET-5 complex were recovered from patients in five European countries, Cuba, and Chile. The five serogroup A isolates with ETs in cluster I consisted of an isolate (D48 with ET-18) from a

TABLE 1. Reactions in three immunoassays of the serotype 21 MAb and the three serotype 4 (4F, 4P, and 4Z) MAbs with 73 serogroup A and B isolates of *N. meningitidis*

Serogroup	Reactions[a] of serotype MAbs in:												No. of isolates
	Dot blotting				ELISA				Immunoblotting[c]				
	21	4F	4P	4Z	21	4F	4P	4Z	21	4F	4P	4Z	
A	+	+	+	−	+	+	+	(+)	+	+	+	−	11
	+	+	+	−	+	+	+	+	+	+	+	−	5
	+	−	−	−	+	−	+	−	+	−	−	−	24
	−	+	(+)	+	−	+	+	+	−	+	−	+	1[d]
	(+)	−	−	−	+	−	−	−	−	−	−	−	1[e]
B	+	+	+	−	+	+	+	(+)	+	+	+	−	2[b]
	−	+	(+)	+	−	+	+	+	−	+	−	+	19
	−	+	+	+	−	+	+	+	−	+	−	+	6
	−	+	−	+	−	+	+	+	−	+	−	+	2
	−	+	−	+	−	+	−	+	−	+	−	+	1
	−	+	(+)	(+)	−	+	−	+	−	+	−	−	1

[a] Weak reactions with the serotype MAbs are shown in parentheses.
[b] Genotypes of these strains are similar to those of some serogroup A strains.
[c] 30 strains representative of different genotypes were immunoblotted.
[d] Strain P53.
[e] Carrier strain D48.

healthy carrier in Norway and four strains of the closely related ETs (ETs 22 through 25) from patients in China (1976 to 1982).

Cluster II consisted of 25 ETs of 37 (92%) serogroup A strains and the three remaining serogroup B strains. Two of the latter had ETs identical to those of serogroup A strains. One was an isolate from Germany with the same ET (ET-42) as three serogroup A strains recovered in Germany at the same time. The other one was a strain from Denmark isolated in 1942 which had the same genotype (ET-45) as the serogroup A reference strain B535 isolated during an epidemic in England in 1941. The remaining serogroup B strain in cluster II was ET-50. Cluster II could be differentiated into the subgroups IIa (ETs 26 through 43) and IIb (ETs 44 through 50) as seen from the dendrogram in Fig. 1.

Dot blotting. The incidences of binding of the serotype 4 and 21 MAbs to the isolates are shown in Table 1. Most of the serogroup A isolates (40 of 42; 95%) had one of two reaction patterns on dot blots: either a distinct binding of the serotype 21 MAb only or of this and the 4F and 4P MAbs. The two deviating strains were the carrier isolate (D48) in cluster I, with a weak serotype 21 reaction, and a strain (P53) in cluster IIb, which was an exception because it did not bind the 21 MAb but reacted with all three serotype 4 MAbs.

The two common MAb binding patterns of the serogroup A isolates were related to their multilocus enzyme genotypes (Fig. 1 and Table 1). The five group A strains in cluster I reacted with the serotype 21 MAb only, as did isolates with ETs 27 through 41 in cluster IIa. All serogroup A isolates in cluster IIb reacted with the 21, 4F, and 4P MAbs, except for P53 (see above) and one strain binding the serotype 21 MAb only. Apart from the latter isolate, all strains with a particular ET had the same MAb reaction pattern.

The two serogroup B isolates with genotypes identical to those of serogroup A strains (ETs 42 and 45) had the same reaction patterns as the serogroup A strains to which they were genetically related (Table 1). Essentially all other serogroup B strains bound the 4F and 4Z MAbs distinctly on dot blots, but not the serotype 21 MAb. The binding of the 4P MAb varied but generally was weak (Table 1). If heat inactivation of the antigens was omitted, the 4P MAb reaction with the cells disappeared. The serotype 21 prototype (ATCC 13077) bound the 21, 4F, and 4P MAbs on dot blots, whereas the serotype 4 prototype (M981) reacted distinctly with the 4F and 4Z MAbs and moderately with the 4P MAb.

ELISA. With few exceptions, the 4F and 21 MAbs had the same reactions in ELISA and dot blotting, but the 4P and 4Z MAbs reacted differently (Table 1). In ELISA, the 4Z MAb also showed a weak or strong binding to the serogroup A strains with ETs 42 through 49 that reacted with the three other serotype MAbs on dot blots, whereas the 4P MAb bound to all but three of the serogroup A and B strains in our collection. The nonreacting isolates, all in cluster I, were the serogroup A carrier strain D48 with ET-18 and two serogroup B patient strains with ET-1 and ET-10.

To investigate why the 4P and 4Z MAbs bound differently in ELISA and dot blotting, the effects of heat inactivation of the meningococcal cells, the use of the detergent Tween 80 in the reagents, and another buffer (reagent set 2; see Materials and Methods) in the ELISA were studied with four strains. The isolates were the reference strains M981 and ATCC 13077 (for MAb reactions on dot blots, see above); strain B453, which reacted on dot blots with the 21 MAb only; and strain 8532, a B:15:P1.3 isolate used as a negative control. As shown in Fig. 2, the bindings of the 21 and 4F MAbs to the plated cells were little affected by the various experimental conditions, in contrast to those of the 4P and 4Z MAbs. With fresh cells and reagent set 2, the ELISA results were similar to the dot blotting results. But if heat-inactivated cells were analyzed with reagent set 2, the reactions of the 4P and 4Z MAbs increased. In the ordinary ELISA method, the bindings of 4Z MAb to ATCC 13077 and 4P MAb to M981 and B453 were amplified by both heat and Tween 80, whereas that of 4Z MAb to M981 was increased only by heat (Fig. 2).

Immunoblotting. The reaction of the serotype MAbs with 30 of the 73 strains, representing various genotypes of the three clusters in the dendrogram (Fig. 1), was studied on immunoblots by using boiled whole-cell suspensions as antigens. The denatured monomeric porins bound the MAbs only in the presence of the detergent Empigen BB. With this method, the 4F and 21 MAbs had the same reactions as in

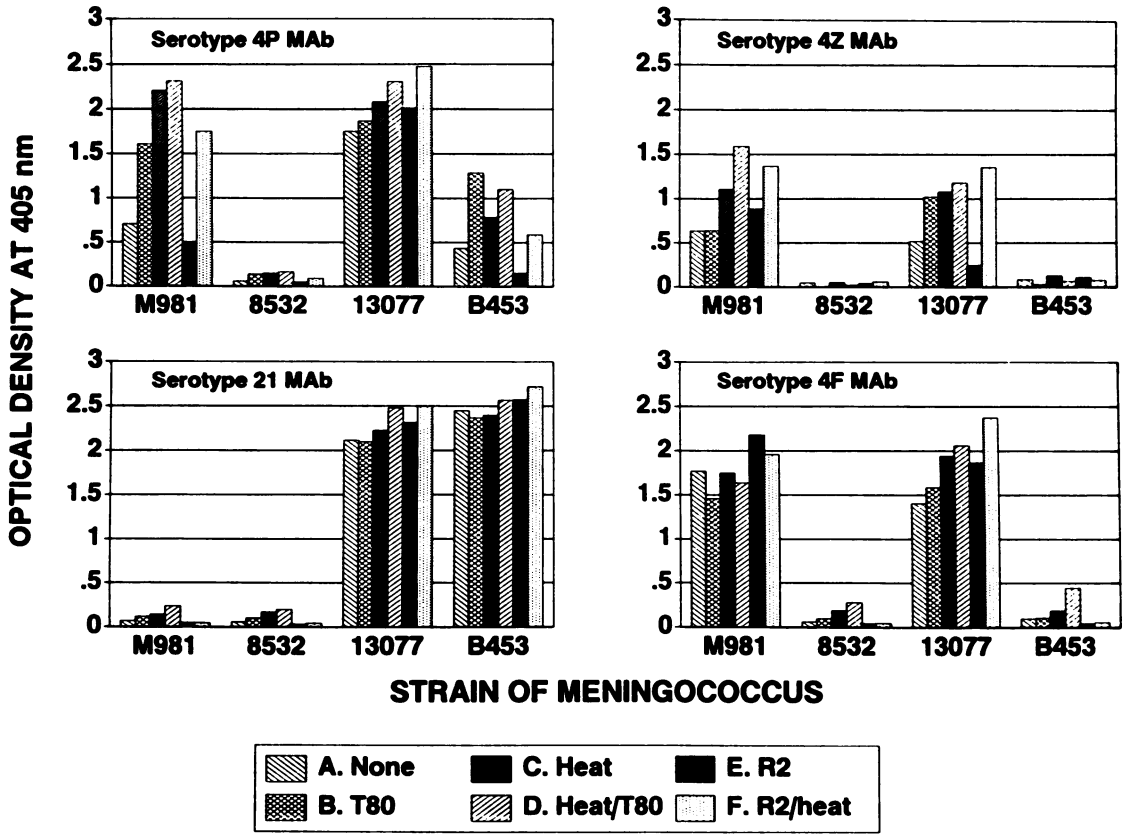

FIG. 2. Effects of different experimental conditions (A to F) on the binding of 21, 4F, 4P, and 4Z MAbs to four meningococcal strains (M981, ATCC 13077, B453, and the negative control, 8532) in ELISA. For A through D, the ELISA procedure and reagents (2, 27) were used except in A, where heat inactivation of the cells was omitted and no Tween 80 was added to the reagents. In B, Tween 80 was used, but the cells were not heat killed; in C, the cells were heat killed but no Tween 80 was used; and in D, the procedure was used without modification. For E and F, a different set of reagents (reagent set 2 [R2]) without detergent was used for washing and diluting the antibodies (see Materials and Methods) and a blocking step was included. In E, cells were not heat killed, and in F they were. The optical densities are means of results in three wells. The MAbs were diluted 1:1,000 for the 4F and 4P MAbs, 1:500 for the 4Z MAb, and 1:2,000 for the 21 MAb.

ELISA and dot blotting and the 4Z MAb reacted as in dot blotting, whereas the 4P MAb showed a different binding pattern (Table 1). No or negligible binding was observed between the latter antibody and the porins of serogroup B strains, which reacted with all three serotype 4 MAbs in ELISA and dot blotting (Fig. 3). Furthermore, 4P MAb did not recognize serogroup A strains that bound this and the 21 MAb in ELISA. The epitope for 4P MAb was, however, present on porins of group A strains (and group B strains of the same genotype) that reacted with this and the 21 and 4F MAbs in the two other immunoassays. The additional binding of the 4Z MAb to these strains in ELISA was not seen on immunoblots (Fig. 3 and Table 1). The porin of the serogroup B strain with ET-1 carried only the epitope for the 4F MAb, and the serogroup A strain D48 with ET-18 showed no serotype 21 reaction on immunoblots (Table 1).

To investigate whether less denatured porins would show another set of epitopes, the reactions of the MAbs with the polymeric forms of the porins were studied after mild dissociation of the two reference strains at 45°C (Fig. 4). The polymeric porin of the serotype 21 reference strain (ATCC 13077) demonstrated detergent-independent binding of the 21, 4F, and 4P MAbs and no binding of the 4Z MAb. In the presence of detergent, to increase the renaturation of the antigens (24), both the polymeric and monomeric porin proteins, as well as a range of antigens in between, reacted with the same MAbs. When the porins were completely denatured by boiling, the high-molecular-weight bands disappeared, and only the immunoreactive band of the monomer was observed in the presence of detergent, as shown in Fig. 3. The polymeric and monomeric porin of the serotype 4 prototype M981 displayed distinct binding of the 4F and 4Z MAbs and negligible and no reaction with the 4P and 21 MAbs, respectively (Fig. 3 and 4). Thus, for each of the strains the polymeric and monomeric porins demonstrated the same reaction patterns with the serotype MAbs.

When Triton X-100 was omitted from the sample buffer during the treatment at 45°C (data not shown), only weak antibody binding to the polymeric porins was observed with immunoblots. These experiments, supported by correspondingly stained SDS gels, indicated that inclusion of Triton X-100 in the buffer during the heat treatment prevented the high-molecular-weight porin from disintegrating into the monomeric form.

SDS-gel electrophoresis of 60 of the meningococcal strains demonstrated that the porins of serogroup A strains, carrying the epitopes for the 21, 4F, and 4P MAbs, might vary a little in molecular weight between 37,000 and 38,000, whereas those with an epitope for the 21 MAb had only slightly lower molecular weights (data not shown). The

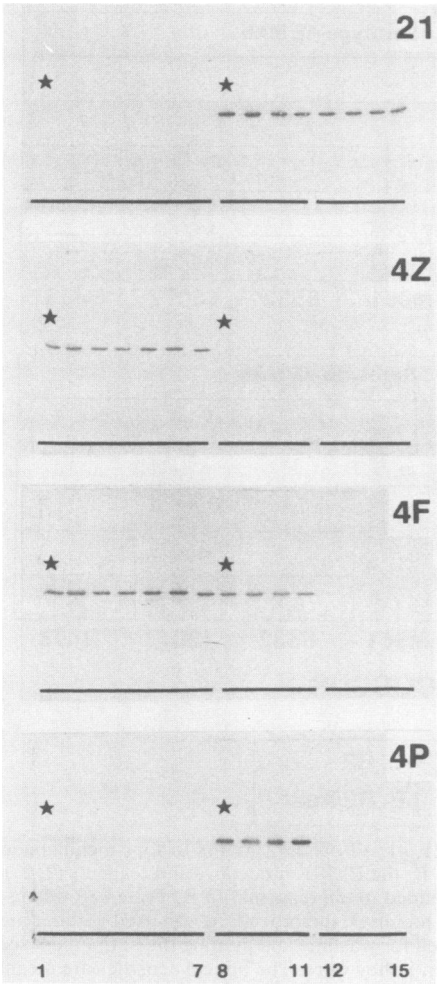

FIG. 3. Immunoblots of meningococcal strains from four identical SDS gels after incubation with the different serotype 4 (4Z, 4F, and 4P) and 21 MAbs. The meningococcal isolates showed the following binding patterns in ELISA: lanes 1 to 7, strains reacted with all three serotype 4 MAbs; lanes 8 to 11, strains reacted with all four serotype 4 and 21 MAbs; and lanes 12 to 15, strains reacted with the 21 and 4P MAbs. The lanes are marked at the bottom of each blot. In lanes 1 and 8 were the reference strains for serotypes 4 (M981) and 21 (ATCC 13077), respectively, indicated by stars. The antigens were boiled in sample buffer with 2-mercaptoethanol before gel electrophoresis, and the blots were incubated with the MAbs in the presence of 0.25% Empigen BB. The serotype 4 MAbs were diluted 1:5,000, and the serotype 21 MAb was diluted 1:20,000.

monomeric porins of serotype 4 serogroup B isolates generally had somewhat higher molecular weights than those of the serogroup A strains. Also, the porins of some serogroup B isolates showed more variation in molecular weight. The two strains in ET-1 and ET-2, M981 and one strain from England that reacted on immunoblots with the 4F MAb only (Table 1), had porins of about 39,000, whereas one strain from Switzerland (94 with ET-8) had a molecular weight of 34,000. Similar variations have been reported previously (1).

DISCUSSION

The aim of this study was to investigate whether the serotype porins of serogroup A meningococci were serotype

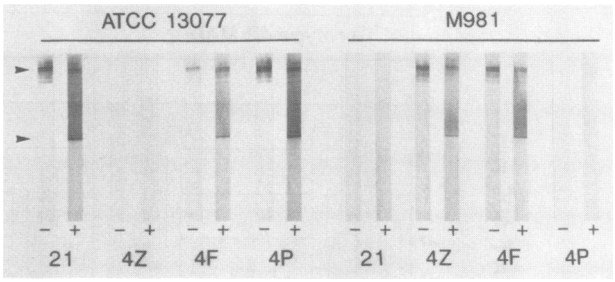

FIG. 4. Immunoblots of the meningococcal prototype strains for serotypes 4 (M981) and 21 (ATCC 13077). Whole-cell suspensions were mildly denatured in 0.5% SDS and 0.5% Triton X-100 at 45°C for 30 min (1a) before SDS-gel electrophoresis. The nitrocellulose blots were incubated with the 21, 4Z, 4F, and 4P MAbs in the presence (+) and absence (−) of 0.25% Empigen BB. The MAbs were diluted as described in the legend to Fig. 3. The top and bottom arrowheads indicate the positions of the polymeric and monomeric form of the class 3 serotype porin, respectively.

21 or similar to the serotype 4 of serogroup B strains. We therefore analyzed the binding of one serotype 21 and three serotype 4 MAbs to genetically characterized meningococci from various parts of the world. Serogroup A strains showed two reaction patterns with the MAbs, which were different from that of the group B strains, implying that the serotypes of serogroup A strains were distinct from the serotype 4 of group B isolates. The use of various immunoassays for serotyping demonstrated that some of the antigen-antibody reactions were observed only with one of the methods; this explained the previous characterizations of serogroup A strains as serotype 4 (1a, 16).

Our results indicated that serogroup A strains generally had one of two different porins. Some strains expressed porins with an epitope for the serotype 21 MAb only, whereas others had porins with epitopes for this antibody as well as the 4F and 4P MAbs. The two porins also had slightly different molecular weights. Only one group A strain in the collection did not react with the serotype 21 MAb. The serotype 21 reaction of serogroup A isolates corresponded to previous results (9), but the use of the other MAbs gave further differentiation of these strains. The porins of serotype 4 serogroup B strains carried epitopes for the 4F and 4Z MAbs but not for 4P and 21 MAbs and demonstrated more variations in molecular weight. Thus, in contrast to previous reports (1a, 6, 16), the data showed that serogroup A strains do not have the same serotype porins as serotype 4 serogroup B strains.

The serotype 4 and 21 MAbs reacted with isolates of distantly related genotypes. However, the blotting results showed that the 4Z and 21 MAbs, but not the 4F and 4P MAbs, could differentiate between most isolates of different genetic lineages (Fig. 1). These two MAbs also discriminated almost completely between serogroups A and B. With few exceptions, the serotype 21 MAb bound to all serogroup A strains in our collection and the 4Z MAb bound to all serotype 4 serogroup B strains, except those with genotypes identical to those of serogroup A strains. The 4F MAb showed binding to both serogroup A and B isolates (Table 1). It reacted with the same serogroup B strains as 4Z MAb and with one of the two subgroups of the serogroup A strains. This subgroup was the only one recognized by the 4P MAb on immunoblots.

A total of 21 strains in our study were from the collection

of serogroup A isolates, described by Olyhoek et al. (15), which were assigned to serotype 4 after the reaction with 4P MAb in ELISA (6). A comparison of their clonal analysis with our blotting data indicated that strains in their clone I carried the serotype 21 epitope only. Clone II also contained such strains and strains reacting with the 21, 4F, and 4P MAbs. Our ETs of clone I and II strains were in cluster IIa. Isolates belonging to their clones III and IV (15) bound the 21, 4F, and 4P MAbs and were in cluster IIb. Recently, Crowe et al. (7) reported that serogroup A Gambian strains in clone IV also reacted with the serotype 21 MAb.

The use of various immunoassays for serotyping showed that the 4P and 4Z MAbs reacted differently in ELISA than with the blotting methods (Table 1). Such method-dependent specificities may be a problem since some laboratories routinely use ELISA and others use dot blotting for typing of meningococci. The binding of 4P MAb in ELISA to essentially all serogroup A and B strains corresponded to previous studies using ELISA and this reagent, which led to the recent serotype 4 designation of serogroup A strains (1a, 16). The serotype 4 assignment of serogroup A strains in those reports was supported by immunoblots of the prototypes ATCC 13077 and M981. However, the reaction in gel immunoradioassay of the 4P MAb with the polymeric porin of M981 (1a) was not observed under our immunoblotting conditions (Fig. 4).

Our studies indicated that the different reactions of the 4P and 4Z MAbs in ELISA and dot blotting were caused by heat inactivation of the cells and exposure to Tween 80 in the ELISA (Fig. 2). As heat-inactivated cells were used as antigens in both methods, but the binding of the 4P and 4Z MAbs differed depending on whether the antigen was adsorbed to nitrocellulose or plastic, the exposure of new epitopes after binding to the plastic surface is likely. The appearance of new antigenic sites after adsorption of proteins to ELISA plates has previously been reported (10, 11, 13).

The different specificities observed for the serotype 4 MAbs might be caused by the various antigens used to prepare the reagents. The 4P MAb was made with outer membrane fractions from three nontypeable serogroup B strains (1a) and the 4F MAb was made with outer membrane vesicles from a strain isolated during an outbreak in Miami (8), while the 21 and 4Z antibodies were prepared with live meningococci (28) from a Brazilian serogroup A strain and a serogroup B strain from Miami, respectively. The use of intact bacteria for immunization of mice may enhance the possibility of obtaining antibodies against determinants exposed on the surface of the organisms only and may also be more relevant with regard to host-bacterium interactions.

The results presented in this study indicated that MAbs selected for serotyping of meningococci should have specificities independent of the assay systems used, give clear-cut negative or positive reactions in the serotype assays, overlap minimally with other serotype MAbs, and bind to major porin epitopes exposed on the viable organisms. Our findings may also have relevance for MAbs used for classification of other microorganisms.

ACKNOWLEDGMENTS

We gratefully acknowledge the gifts of MAbs from C. E. Frasch and J. T. Poolman and serogroup A strains from M. Achtman. Karin Bolstad is thanked for excellent technical assistance.

D.A.C. was supported by grant 13.48.07-026 from the Norwegian Council for Science and the Humanities.

REFERENCES

1. **Abdillahi, H.** 1988. Ph.D. thesis. State University of Utrecht, Utrecht, The Netherlands.
1a. **Abdillahi, H., B. A. Crowe, M. Achtman, and J. T. Poolman.** 1988. Two monoclonal antibodies specific for serotype 4 antigen of *Neisseria meningitidis*. Eur. J. Clin. Microbiol. Infect. Dis. **7**:293–296.
2. **Abdillahi, H., and J. T. Poolman.** 1987. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. FEMS Microbiol. Lett. **48**:367–371.
3. **Abdillahi, H., and J. T. Poolman.** 1988. Typing of group-B *Neisseria meningitidis* with monoclonal antibodies in the whole-cell ELISA. J. Med. Microbiol. **26**:177–180.
4. **Caugant, D. A., L. O. Frøholm, K. Bøvre, E. Holten, C. E. Frasch, L. F. Mocca, W. D. Zollinger, and R. K. Selander.** 1986. Intercontinental spread of a genetically distinctive complex of clones of *Neisseria meningitidis* causing epidemic disease. Proc. Natl. Acad. Sci. USA **83**:4927–4931.
5. **Caugant, D. A., F. Mocca, C. E. Frasch, L. O. Frøholm, W. D. Zollinger, and R. K. Selander.** 1987. Genetic structure of *Neisseria meningitidis* populations in relation to serogroup, serotype, and outer membrane protein pattern. J. Bacteriol. **169**: 2781–2792.
6. **Crowe, B. A., H. Abdillahi, J. T. Poolman, and M. Achtman.** 1988. Correlation of serological typing and clonal typing methods for *Neisseria meningitidis* sero-group A. J. Med. Microbiol. **26**:183–184.
7. **Crowe, B. A., R. A. Wall, B. Kusecek, B. Neumann, T. Olyhoek, H. Abdillahi, M. Hassan-King, B. M. Greenwood, J. T. Poolman, and M. Achtman.** 1989. Clonal and variable properties of *Neisseria meningitidis* isolated from cases and carriers during and after an epidemic in The Gambia, West Africa. J. Infect. Dis. **159**:686–700.
8. **Frasch, C. E., L. F. Mocca, and A. B. Karpas.** 1988. Appearance of new strains associated with group B meningococcal disease and their use for rapid vaccine development, p. 97–104. *In* J. T. Poolman et al. (ed.), Gonococci and meningococci. Kluwer Academic Publishers, Dordrecht, The Netherlands.
9. **Frasch, C. E., W. D. Zollinger, and J. T. Poolman.** 1985. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev. Infect. Dis. **7**:504–510.
10. **Friguet, B., L. Djavadi-Ohaniance, and M. E. Goldberg.** 1984. Some monoclonal antibodies raised with a native protein bind preferentially to the denatured antigen. Mol. Immunol. **21**:673–677.
11. **Hollander, Z., and E. Katchalski-Katzir.** 1986. Use of monoclonal antibodies to detect conformational alterations in lactate dehydrogenase isoenzyme 5 on heat denaturation and on adsorption to polystyrene plates. Mol. Immunol. **23**:927–933.
12. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
13. **Miller, K. F., D. J. Bolt, and R. A. Goldsby.** 1983. A rapid solution-phase screening technique for hybridoma culture supernatants using radiolabeled antigen and a solid-phase immunoadsorbent. J. Immunol. Methods **59**:277–280.
14. **Mocca, L. F., and C. E. Frasch.** 1982. Sodium dodecyl sulfate-polyacrylamide gel typing system for characterization of *Neisseria meningitidis* isolates. J. Clin. Microbiol. **16**:240–244.
15. **Olyhoek, T., B. A. Crowe, and M. Achtman.** 1987. Clonal population structure of *Neisseria meningitidis* serogroup A isolated from epidemics and pandemics between 1915 and 1983. Rev. Infect. Dis. **9**:665–692.
16. **Poolman, J. T., and H. Abdillahi.** 1988. Outer membrane protein serosubtyping of *Neisseria meningitidis*. Eur. J. Clin. Microbiol. Infect. Dis. **7**:291–292.
17. **Poolman, J. T., and T. M. Buchanan.** 1983. Monoclonal antibodies against meningococcal outer membrane proteins. Med. Trop. **43**:139–142.
18. **Poolman, J. T., S. de Marie, and H. C. Zanen.** 1980. Variability of low-molecular-weight, heat-modifiable outer membrane proteins of *Neisseria meningitidis*. Infect. Immun. **30**:642–648.

19. **Rosenqvist, E., E. Wedege, E. A. Høiby, and L. O. Frøholm.** 1990. Serogroup determination of *Neisseria meningitidis* by whole-cell ELISA, dot-blotting and agglutination. Acta Pathol. Microbiol. Immunol. Scand. **98**:501–506.
20. **Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam.** 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl. Environ. Microbiol. **51**:873–884.
21. **Sippel, J. E., and A. Quan.** 1977. Homogeneity of protein serotype antigens in *Neisseria meningitidis* group A. Infect. Immun. **16**:623–627.
22. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman & Co., San Francisco.
23. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
24. **Wedege, E., K. Bryn, and L. O. Frøholm.** 1988. Restoration of antibody binding to blotted meningococcal outer membrane proteins using various detergents. J. Immunol. Methods **113**:51–59.
25. **Wedege, E., and L. O. Frøholm.** 1986. Human antibody response to a group B serotype 2a meningococcal vaccine determined by immunoblotting. Infect. Immun. **51**:571–578.
26. **Wedege, E., E. A. Høiby, E. Rosenqvist, and L. O. Frøholm.** 1990. Serotyping and subtyping of *Neisseria meningitidis* isolates by coagglutination, dot-blotting and ELISA. J. Med. Microbiol. **31**:195–201.
27. **Zollinger, W. D., and R. E. Mandrell.** 1980. Type-specific antigens of group A *Neisseria meningitidis*: lipopolysaccharide and heat-modifiable outer membrane proteins. Infect. Immun. **28**:451–458.
28. **Zollinger, W. D., E. E. Moran, H. Connelly, R. E. Mandrell, and B. Brandt.** 1984. Monoclonal antibodies to serotype 2 and serotype 15 outer membrane proteins of *Neisseria meningitidis* and their use in serotyping. Infect. Immun. **46**:260–266.