

## Outer Membrane Protein Serotyping of *Neisseria meningitidis*

J. T. Poolman\*, H. Abdillahi

A system for typing *Neisseria meningitidis* has been developed which uses monoclonal antibodies against two separate classes of outer membrane proteins (class 1 and class 2/3) in addition to capsular polysaccharide serogrouping. It was shown that class 1 outer membrane protein subtypes are common to meningococcal reference strains of different serotypes. Application of the system to 50 group B meningococcal patient isolates revealed that 75 % could be categorized as class 1 subtypes. The typing system has potential usefulness in epidemiological surveillance and vaccine development.

Meningococci express several outer membrane proteins (OMPs), lipo-oligosaccharides and pili. The biological function of class 1 OMP is unknown, class 2/3 OMP appears to function as a porin (1), class 4 OMP reveals partial homology with *Escherichia coli* OMP A proteins (2), and class 5 OMP probably has an adhesion function like gonococcal p II (3). Monoclonal antibodies against several outer membrane components have been found to have strong bactericidal activity, particularly against class 1 OMPs and lipo-oligosaccharides (4).

These surface antigens reveal serotype heterogeneity and the occurrence among patient isolates of the various serotype antigens needs to be documented. Ideally, the bacterial markers used for epidemiological surveillance and vaccine development are the same. Examination of the various properties of meningococcal outer membrane components has resulted in the proposal of a serotype nomenclature (5) which combines the typing features of class 2/3 OMPs, class 1 OMPs, class 5 OMPs and lipo-oligosaccharides. Meningococcal lipo-oligosaccharides and class 5 OMPs reveal high intra-strain variability (6) which limits their usefulness in investigations of spread of meningococci. In addition, the intrinsic toxic properties of lipo-oligosaccharides limit their potential use in the development of a vaccine.

This paper reports on the current status and application of a meningococcal serotyping system using monoclonal antibodies selected for their bactericidal

activity to characterize the class 1 OMP subtype of class 2/3 OMP serotype reference strains.

**Materials and Methods.** Meningococci were selected from culture collections as already reported (7) and serotyped by a whole-cell ELISA as described elsewhere (7). The preparation of monoclonal antibodies and the characterization by gel-immuno-radio-assay were performed as described previously (8). The procedure for determination of bactericidal activity of monoclonal antibodies was as described elsewhere (4).

**Results and Discussion.** Table 1 shows the relationship between the class 2/3 OMP serotype system (5) and the class 1 OMP subtype antigens as determined using monoclonal antibodies. Class 1 OMP subtype antigens were common to different class 2/3 OMP serotype reference strains: subtype P1.1 was found in serotype 1, 5, 8, 16, 20 reference strains; subtype P1.2 in serotype 2a, 2b, 2c, 3 reference strains; and subtype P1.6 in serotype 6, 14, 18, 19 reference strains. Serotype 4 and 11 reference strains did not express detectable class 1 OMPs. The serotype 21 serogroup A reference strain appears to belong to serotype 4 (7). At present a few subtype class 1 OMPs can still not be characterized because monoclonal antibodies are not yet available against these proteins.

Table 2 shows that use of five P1-subtype monoclonal antibodies, P1.1, P1.2, P1.9, P1.15, P1.16, enabled typing of approximately 75 % of 50 group B meningococcal strains obtained in various countries at various times.

Table 1: Current status of the meningococcal outer membrane protein serotyping system.

Class 2/3 OMP serotype	Class 1 OMP subtype	Prototype strain	
		Designation	Serogroup
1	P1.1	M1080	B
2A	P1.2	B16B6	B
2B	P1.2	2996	B
2C	P1.2	2396	NG
3	P1.2	126E	C
4	P1.-	M981	B
5	P1.1	M992	B
6	P1.6	M990	B
8	P1.1	M978	B
9	P1.9	M982	B
11	P1.-	M136	B
12	P1.16	S-3032	B
14	P1.6	S-3446	B
15	P1.15	H355	B
16	P1.1	60E	C
17	P1.?	6557	B
18	P1.6	1901	B
19	P1.6	6940	B
20	P1.1	35E	C
21 (4)	P1.-	ATCC 13077	A

**Table 2:** Analysis of 50 group B meningococci from various sources for the presence of class 1 outer membrane protein subtype antigens.

Country	No. of strains	Subtype antigens					No. of positive strains
		P1.1	P1.2	P1.9	P1.15	P1.16	
FRG	5	—	2	—	1	—	3
Finland	3	—	1	—	—	2	3
Spain	10	—	2	1	1	3	7
Netherlands	10	2	—	1	2	1	6
Australia	10	2	—	4	—	—	6
Denmark	5	1	1	1	—	—	3
China/Hongkong	7	1	5	1	—	—	7
Total	50	6	11	8	4	6	35

This study reports on the class 1 OMP subtype distribution of serotype reference strains of *Neisseria meningitidis*. It was found that subtypes are common to strains having different serotype characteristics. This result was confirmed by testing a number of group B meningococci obtained in various countries at different times. Approximately 75% of these group B meningococcal strains could be categorized as class 1 OMP subtypes using five class 1 OMP specific monoclonal antibodies (P1.1, P1.2, P1.9, P1.15, P1.16). This figure rose above 90% when more class 1 OMP monoclonal antibodies were added to the system (unpublished data).

The establishment of a class 1 OMP typing system for meningococci should enable investigation of the spread of meningococci and at the same time provide indications on the possibility of developing a meningococcal OMP vaccine. An important property of monoclonal antibodies against class 1 OMPs used for typing is their bactericidal activity, which allows linkage of surveillance and vaccine development.

The recent demonstration of spread of the epidemic B:2b:P1.2 and B:15:P1.16 (serogroup: class 2/3 OMP: class 1 OMP) meningococci and the change over time (9) indicates the potential usefulness of the proposed system.

## References

- Lynch, E. C., Blake, M. S., Gotschlich, E. C., Mauro, A.: Studies on porins spontaneously transferred from whole cells and reconstituted from purified proteins of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Biophysical Journal* 1984, 45: 104–107.
- Gotschlich, E. C., Seiff, M., Blake, M. S.: The DNA sequence of the structural gene of gonococcal protein III and the flanking region containing a repetitive sequence. *Journal of Experimental Medicine* 1987, 165: 471–482.
- King, G. J., Swanson, J.: Studies on gonococcus infection. XV: Identification of surface proteins of *Neisseria gonorrhoeae* correlated with leukocyte association. *Infection and Immunity* 1978, 11: 575–584.
- Poolman, J. T., Wientjes, F. B., Hopman, C. T. P., Zanen, H. C.: Influence of the length of LPS molecules on the surface exposure of class 1 and 2 outer membrane proteins of *Neisseria meningitidis* strain 2996 (B: 2b: P1.2). In: Schoonik, G. K. (ed.): *Pathogenic Neisseria*. American Society for Microbiology, Washington, DC, 1985, p. 562–570.
- Frasch, C. E., Zollinger, W. D., Poolman, J. T.: Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. *Reviews of Infectious Diseases* 1985, 7: 504–510.
- Poolman, J. T., Hopman, C. T. P., Zanen, H. C.: Colony variants of *Neisseria meningitidis* strain 2996 (B:2b:P1.2). Influence of class 5 outer membrane proteins and lipopolysaccharides. *Journal of Medical Microbiology* 1985, 12: 203–209.
- Abdillahi, H., Crowe, B. A., Achtman, M., Poolman, J. T.: Two monoclonal antibodies specific for serotype 4 antigens of *Neisseria meningitidis*. *European Journal of Clinical Microbiology and Infectious Diseases* 1988, 7: 293–296.
- Poolman, J. T., Buchanan, T. M.: Monoclonal antibody activity against native and denatured forms of gonococcal outer membrane proteins as detected within ultrathin, longitudinal slices of polyacrylamide gels. *Journal of Immunological Methods* 1984, 75: 265–274.
- Poolman, J. T., Lind, I., Jonsdottir, K., Froholm, L. O., Jones, D. M., Zanen, H. C.: The occurrence of serotypes 2b and 15 among group B meningococci in North-West Europe. *Lancet*, 1986, ii: 555–558.