Monoclonal antibody recognition of members of the meningococcal P1.10 variable region family: implications for serological typing and vaccine design

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Identification of antigenic variants of the PorA protein of *Neisseria meningitidis* with specific mAbs (serosubtyping) is used in meningococcal strain characterization and the resultant data has been exploited in the design of novel multivalent vaccines against this important pathogen. The reactivity of the P1.10 serosubtyping mAb MN20F4.17 with eight members of the meningococcal P1.10 variable region (VR) family (prototype P1.10 and variants P1.10a–P1.10g), identified by nucleotide sequence analysis of *porA* genes, was investigated. Analysis of overlapping synthetic octapeptides by ELISA demonstrated that the peptide sequence, QNQRPTL, present only in the prototype P1.10, was sufficient for binding of the mAb. A linear peptide of 14 amino acids, containing the minimum epitope, inhibited binding of mAb MN20F4.17 to whole cells in a competitive ELISA. This binding was weak compared with a tethered peptide or the native protein. In whole-cell ELISA or dot-blot assays using low concentrations of mAb MN20F4.17 only the prototype P1.10 was detected. However, when higher concentrations of antibody were used the prototype P1.10 was detected, together with variants P1.10a, P1.10c and P1.10e by whole-cell ELISA and P1.10a and P1.10c by the immunoblot technique. The variants P1.10b, P1.10d, P1.10f and P1.10g showed no reactivity with mAb under any of the conditions tested. A survey of the *porA* genes in serogroup B and C strains revealed that the P1.10a variant, rather than the prototype P1.10, was the most common member of the P1.10 VR family in England and Wales. These data illustrate: (i) the problems associated with epidemiological analyses that rely solely on monoclonal antibodies; (ii) the importance of using defined assay conditions for serosubtyping; and (iii) that genetical analyses provide more reliable information than serological data based on murine reagents for the design of candidate vaccines that include PorA.

Keywords: *Neisseria meningitidis*, PorA, monoclonal antibody, serosubtyping, antigenic variation

INTRODUCTION

Bacterial meningitis and septicaemia, caused by the human pathogen *Neisseria meningitidis*, are characterized by rapid onset and a high mortality rate (Peltola, 1983; Bannister, 1988; Hart & Rogers, 1993). The management of these

diseases, which affect only a small proportion of meningococcal carriers, requires swift diagnosis and comprehensive surveillance (Broome, 1986; Schwartz *et al.*, 1989; Caugant *et al.*, 1994). Murine mAbs are employed for the routine epidemiological characterization of meningococci by the identification of antigenic variants of the PorA protein, a porin (Abdillahi & Poolman, 1987, 1988a). This protein is also a major component of several anti-meningococcal vaccines included in clinical trials (Fine,

Abbreviation: VR, variable region.

1991; Sierra *et al.*, 1991; van der Ley *et al.*, 1995). However, many isolates fail to react with the current set of mAbs and new approaches are required to identify these strains for epidemiological analysis and to provide data for vaccine design and assessment.

The application of molecular techniques, such as direct nucleotide sequence determination, permits the characterization of meningococcal PorA variants by deduced amino acid sequence rather than by antibody reactivity (Suker *et al.*, 1994). The term 'subtype' is best used to refer to PorA proteins characterized by the deduction of their amino acid sequences from nucleotide sequence data and 'serosubtype' to indicate characterization solely by mAbs. Comparative and immunological studies have shown that subtypes reside in two variable regions (VRs), designated VR1 and VR2, of PorA which correspond to surface loops I and IV of the proposed porin structure (Barlow *et al.*, 1989; McGuinness *et al.*, 1990, 1993; Butcher *et al.*, 1991; van der Ley & Poolman, 1991; van der Ley *et al.*, 1991; Maiden *et al.*, 1991; Suker *et al.*, 1994). Distinct VR1 and VR2 peptide sequences are assigned arbitrary numbers and confer a dual subtype designation given after the prefix 'P1.', e.g. P1.5,10, where 5 is located in VR1 and 10 in VR2.

The accumulation of mutations in the subtype-encoding regions of the *porA* gene results in VR 'families' that comprise related peptide sequences (Suker *et al.*, 1994). The minor variants are distinguished by the addition of a lower case letter after the subtype designation; e.g., P1.10a. Continuous peptide epitopes within the subtype sequences that are recognized by the serosubtyping mAbs have been defined for a number of the VR families (McGuinness *et al.*, 1990, 1993). However, small amino acid changes in the VR families may result in the loss or sequestration of these epitopes in some variants (McGuinness *et al.*, 1991, 1993; Wedege *et al.*, 1993).

The present work was undertaken to define the reactivity of members of the P1.10 VR family with serosubtyping mAb MN20F4.17. The P1.10 family was chosen as it is widely distributed in meningococci and the prototype P1.10 sequence is included in a multivalent PorA vaccine (van der Ley *et al.*, 1995). The minimum amino acid sequence recognized by the P1.10 serosubtyping mAb MN20F4.17 was defined and the immunological reactivities of this antibody with eight members of the P1.10 VR family were compared by ELISA and immunoblotting. The relative contributions of amino acid sequence and epitope conformation in serosubtype recognition were investigated by immunoassay of membrane preparations subjected to boiling in SDS and by a competitive ELISA of a linear peptide with protein in its native conformation. Finally, the distribution of members of the P1.10 VR family among serotyped P1.10 and non-serotypeable serogroup B and C strains was determined by comparative analyses of *porA* genes. These data show that for the P1.10 VR family serosubtyping with mAb underestimates the antigenic diversity of PorA. We conclude that multivalent PorA vaccines designed on the basis of serosubtype data alone may not necessarily

contain the most appropriate variant of a given VR family.

METHODS

Bacterial strains. Meningococcal strains used in this study were provided by the Public Health Laboratory, Withington Hospital, Manchester and by the Max-Planck Institut für molekulare Genetik, Berlin. The nine strains in Table 1 were identified in surveys of 288 meningococcal isolates (55 strains in Suker *et al.*, 1994; 233 strains in I. M. Feavers, A. J. Fox, S. Gray, D. M. Jones, J. Suker & M. C. J. Maiden, unpublished results). The strains from these surveys were also used for the assessment of *porA* diversity. Strain Z1388 was chosen as a negative control strain as its *porA* gene is identical to that encoding the prototype P1.10 (Z1040) except for an 87 bp deletion which removes VR2 (Suker *et al.*, 1994). The VR2 sequences of the eight P1.10 variant strains were confirmed by direct nucleotide sequence analysis prior to this study using the method of Suker *et al.* (1994). The expression of the *porA* gene by each of the nine strains was tested by separation of membrane protein samples by SDS-PAGE. All of the strains produced a PorA protein visible by Coomassie Brilliant Blue staining. A further 13 recent clinical isolates of serosubtype P1.10, serogroup B and C strains (strain numbers, 2651–2663) were obtained from the Public Health Laboratory, Manchester and were unique to this study.

Propagation of meningococcal strains and preparation of DNA. Meningococcal strains (Table 1), were maintained at -70°C in Müller–Hinton broth with 50% (v/v) 'freezing mix' [10% (v/v) glycerol; 3 mM sodium citrate; 1 mM ammonium sulphate; 1 mM magnesium sulphate; 100 mM potassium phosphate, pH 6.6]. The bacteria were propagated on heated blood (chocolate) agar at 37°C in a 5% (v/v) CO_2 atmosphere for 8–16 h. Preparation of DNA from meningococcal strains was performed using an Isoquick Kit (MicroProbe) by the rapid DNA extraction protocol provided by the manufacturer. Cells were suspended in 500 μl sample buffer (MicroProbe) to approximately 10^8 cells ml^{-1} . Larger scale preparation of DNA was done by scraping the growth from five chocolate agar plates and resuspending the bacteria in 5 ml TE buffer (10 mM Tris/HCl containing 1 mM EDTA, pH 8.0) to which 5 ml 100 mM Tris/HCl, pH 7.5, containing 100 mM NaCl, 10 mM EDTA and 1% (v/v) Sarkosyl was added. Proteinase K was added to a final concentration of $100\text{ }\mu\text{g ml}^{-1}$ and the samples were incubated at 55°C for 2 h. The mixture was then extracted once with phenol, once with phenol/chloroform and once with chloroform. Boiled RNase A (final concentration $100\text{ }\mu\text{g ml}^{-1}$) was added to the extract which was incubated at room temperature for 2 h. The samples were again extracted with phenol, phenol/chloroform and chloroform and dialysed overnight against two changes of $0.1\times$ TE buffer. The DNA concentration and purity were estimated spectrophotometrically before use.

PCR. Reaction components were as follows: 50 ng template DNA μl^{-1} ; 10 mM Tris/HCl, pH 8.0; 50 mM KCl; 1.5 mM MgCl_2 ; 0.001% gelatin; 200 μM of each of dATP, dCTP, dGTP and dTTP; 2 μM of each of the required primers and 0.5 U Taq polymerase (Amplitaq, Cetus). The components were incubated for 30 cycles in a programmable heat block (PHC-2, Techne Instruments) for 2 min at 94°C , 2 min at 68°C or 70°C and 3 min at 72°C . At the end of 30 cycles, the reaction mixtures were incubated for 3 min at 72°C .

Gene fingerprinting by chain termination. Fingerprints from PCR products were produced by the method of Suker *et al.* (1994). Dideoxy termination reactions were run on adjacent tracks of polyacrylamide gels and compared. For example, 'T-

Table 1. Characteristics of nine *N. meningitidis* strains used in this study

Serogroup, serotype and serosubtype assigned at source. NT, Not typable.

Strain number	Country of isolation	Source*	Serogroup†	Serotype	Serosubtype‡	Subtype§	Deduced amino acid sequence in VR2 of P1.10 subtype variant
Z1040	Morocco	MPIG	A	4,21	P1.10	5c,10	HFVQNKQNRPTLVP
2400	UK (Epsom)	PHL	C	2b	NT	5a,10a	HFVQNKQNPPTLVP
2401	UK (Romford)	PHL	NG	NT	NT	5,10b	HFVQDKKGQPTLVP
2376	UK (Brighton)	PHL	C	2a	NT	5,10c	HFVQNKQNPPTLVP
Z4272	USA	MPIG	C	2a	P1.5,2	5a,10d	HFVQNKQNKQNPPTLVP
Z4063	China	MPIG	A	4	NT	7c,10e	HFVQNKQSRPTLVP
2403	UK (Broadgreen)	PHL	NG	NT	NT	5a,10f	HFVQNKQNPPTLVP
2413	UK (Rhyll)	PHL	B	NT	P1.5	5,10g	HFVQNKQNPPTLVP
Z1388	Algeria	MPIG	A	4,21	NT	5c, del	Deleted

* MPIG, Max-Planck Institut für molekulare Genetik (courtesy of Dr M. Achtman); PHL, Manchester Public Health Laboratory (courtesy of Dr A. J. Fox).

† NG, Not groupable.

‡ Determined by reactivity with mAb.

§ VR loop names, defined by nucleotide sequence analysis (Suker *et al.*, 1994; I. M. Feavers, A. J. Fox, S. Gray, D. M. Jones, J. Suker & M. C. J. Maiden, unpublished results).

track' fingerprints were produced by running dideoxythymidine reactions in adjacent lanes. Separate fingerprints were determined for extension reactions terminated with dideoxythymidine, dideoxyguanine and dideoxycytosine.

Membrane preparation and protein analysis. Membranes of *N. meningitidis* were prepared by a modification of the sphaeroplast lysis method (Witholt *et al.*, 1976). A single colony from a chocolate agar plate was inoculated into 5 ml Müller–Hinton broth and grown for 6 h at 37 °C until an OD₆₀₀ of 0.6 (approx. 5×10^8 cells ml⁻¹) was obtained. Cells from 4 ml culture were harvested and resuspended in 200 µl 200 mM Tris/HCl containing 1 mM EDTA, pH 8.0. To this suspension was added 200 µl 200 mM Tris/HCl containing 1 M sucrose and 1 mM EDTA. After mixing for 90 s, 24 µg egg white lysozyme was added followed by 400 µl deionized water after a further 45 s. The suspension was incubated at room temperature with gentle mixing for 30 min. The sphaeroplasts formed were collected by centrifugation at 40 000 *g* for 20 min at 20 °C, and lysed by the addition of 800 µl ice-cold deionized water. The membranes were collected by centrifugation as above and resuspended in 400 µl deionized water. Protein concentrations were estimated by the bicinchoninic acid method using reagents from a BCA kit (Pierce) with BSA as a standard. Membrane proteins were separated on a 12.5% (w/v) acrylamide gel and visualized by staining with Coomassie Brilliant Blue.

Source of mAb MN20F4.17. The mAb was used in the form of ascitic fluid, collected from pristane-treated mice after intraperitoneal injection with cells of the hybridoma cell line MN20F4.17, provided by Dr J. T. Poolman of the National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands (Poolman *et al.*, 1995). Ascitic fluid was stored at -20 °C. Purified antibody was obtained from the ascitic fluid by precipitation with 45% (w/v) ammonium sulphate solution followed by separation on a protein G column.

ELISA. ELISAs were carried out by a modified version of the method of Abdillahi & Poolman (1987). Chocolate agar plates were spread with 100 µl cells from frozen stocks and incubated

at 37 °C in 5% CO₂ atmosphere overnight. For each strain, 5 ml Müller–Hinton broth was inoculated and incubated at 37 °C in a shaking incubator for 8 h or until an OD₆₀₀ of 0.6 was obtained. Cells were harvested from 3 ml broth culture by centrifugation for 10 min in a microfuge and resuspended in 1 ml PBS containing 0.05% Tween 20. The cell suspensions were heated at 55 °C for 30 min to kill the cells and then diluted 1:10 in PBS (1.6×10^8 cells ml⁻¹). Diluted cell suspensions were stored at 4 °C for up to 7 d. For ELISA, Nunc 96-well flat-bottomed microtitre plates were freshly coated for each test with 100 µl per well of the cell suspension and allowed to dry at 37 °C overnight. The plates were washed three times with 0.025% Tween 20. Decimal dilutions of mAb MN20F4.17 (100 µl per well) were added to the plate which was incubated at 37 °C for 1 h. The plate was again washed three times with 0.025% Tween 20. Secondary conjugate (Protein A-peroxidase, 100 µl per well) diluted 1:2500 in PBS containing 0.5% BSA and 0.01% Tween 20, was added and the plate incubated at 37 °C for 1 h. The plate was finally washed with 0.025% Tween 20. ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt, 0.5 mg ml⁻¹] was made up in 0.05 M citric acid, pH 4.0, containing 0.006% hydrogen peroxide and added to the plate (150 µl per well). The colour was allowed to develop for 10 min and the A₄₀₅ was measured. For the inhibition ELISA, 100 µl aliquots of purified mAb MN20F4.17 (either 55 or 550 ng ml⁻¹) were mixed with 100 µl aliquots 0–10 mg peptide ml⁻¹ before addition to the whole-cell-coated plate.

Dot-immunoblotting. Membrane samples were prepared as above and various dilutions made. For non-denatured samples, dilutions were made in PBS; for denatured samples, dilutions were made in transfer buffer minus methanol [25 mM Tris/HCl containing 0.19 M glycine and 0.1% SDS] and boiled for 5 min. A nitrocellulose membrane, presoaked in PBS, was placed in a dot-blot manifold (Bio-Rad), 100 µl samples were applied to the wells and drawn on to the membrane under vacuum. The membrane was cut in half; the portion with non-denatured samples was rinsed in PBS, the portion with denatured samples was rinsed in transfer buffer containing 20% (v/v) methanol to

remove traces of SDS. Both filters were blocked in PBS containing 5% (w/v) powdered milk for 1 h at 25 °C and incubated for 16 h with dilutions of mAb MN20F4.17 in PBS/milk (usually 1:100). After rinsing in several changes of PBS, the filters were incubated for 1 h with Protein A-peroxidase conjugate, diluted 1:1000 in PBS/milk. Following further washes in PBS, dots were visualized by immersing the filters in 0.35 mM 4-chloronaphthol in 16% methanol and 20 mM Tris/HCl, pH 7.4.

Epitope scanning. A set of synthetic peptides was synthesized on the tips of derivatized polyethylene pins in the configuration of a 96-well microtitre plate using the Pin Technology method [Cambridge Research Biochemicals; Geysen *et al.* (1987)]. Each peptide was eight amino acids long and overlapped with the next consecutive peptide by seven amino acids. The synthesis schedule (General Net strategy) was designed so that all known variants of the P1.10 epitope were covered with an overlap of one amino acid. The peptide-bearing pins were blocked for 16 h in 2% (w/v) BSA at 4 °C and then reacted in an ELISA with purified P1.10 antibody using 440 ng IgG per peptide. Anti-mouse IgG-peroxidase conjugate was used as a secondary antibody and binding was detected with ABTS as a substrate. A_{405} was measured and plotted against peptide sequence.

RESULTS

P1.10 variants show different reactivities with mAb MN20F4.17

The reactivities of nine meningococcal strains (Table 1) with mAb MN20F4.17 were assessed by ELISA and immunoblotting. When high concentrations of mAb MN20F4.17 were used (1:10 dilution of ascitic fluid containing mAb or 20 µg purified IgG per well), strains expressing four of the variant PorA proteins examined, prototype P1.10, P1.10a, P1.10c and P1.10e, bound antibody at levels detectable in whole-cell ELISA. At lower antibody concentrations only strain Z1040, expressing the prototype P1.10, bound antibody at levels detectable by this assay (Fig. 1).

Fig. 1. Detection of P1.10 variants by whole-cell ELISA using mAb MN20F4.17. Mean absorbance readings from duplicate samples are plotted against dilutions of ascitic fluid containing mAb MN20F4.17 binding to cells expressing the prototype P1.10 (○) and each of the variants P1.10a (●), P1.10c (□) and P1.10e (■). The background reading from mAb-binding to whole cells of the P1.10-deleted negative control strain (Z1388) has been subtracted, hence variants P1.10b, P1.10d, P1.10f and P1.10g do not appear on the graph as binding to these strains was not above background levels under these conditions.

Fig. 2 Immunoblot of members of the P1.10 variable region family with the P1.10 specific mAb, MN20F4.17. Filters were prepared in a dot-blot manifold using doubling dilutions of membrane samples (6–50 µg total protein per dot) from strains expressing PorA proteins with the prototype P1.10 and variants P1.10a–P1.10g. (a) Non-denaturing conditions, PBS was used for dilution of samples and to wash the filter following transfer; (b) denaturing conditions, SDS-containing buffer was used for dilution and boiling of samples and to wash the filter following transfer. Each filter was probed with ascitic fluid containing mAb MN20F4.17 at a dilution of 1:100.

Immunoblotting experiments showed that mAb MN20F4.17 bound to native membrane samples of strains expressing the prototype P1.10, P1.10a and P1.10c, although the signals obtained with the variant strains were weaker than that obtained with the prototype strain (Fig. 2a). When membrane samples boiled in SDS-containing buffer were used, only membranes from the strain expressing the prototype P1.10 sequence reacted with the antibody at detectable levels (Fig. 2b).

In whole-cell ELISA, the prototype P1.10 was detected by up to 1:10000 dilution of ascitic fluid (Fig. 1) or 2 ng purified IgG per well (data not shown). The P1.10 variants which differed from the prototype P1.10 by only one amino acid (P1.10a, P1.10c and P1.10e) were only reliably detected at 1:10 dilution of mAb MN20F4.17. Four variants were never detected by the mAb; these differed from the prototype sequence by either two (variant P1.10g), four (variants P1.10b and P1.10d) or seven (variant P1.10f) amino acids (Table 1).

Amino acid sequence QNQRPTL identified as the minimum P1.10 epitope reactive with mAb MN20F4.17

The minimum amino acid sequence responsible for the P1.10 epitope was defined by means of a set of overlapping peptides synthesized on the tips of polyethylene pins. Based on the amino acid sequences of the eight variants of

Fig. 3. Localization of the minimum epitope required for P1.10 subtype specificity of mAb MN20F4.17. A set of tethered overlapping synthetic peptides of eight amino acids in length, differing by one amino acid and based on the sequences of the eight P1.10 variants, were reacted in ELISA with purified P1.10 antibody using 440 ng IgG per peptide. The amino acid sequences of the peptides (printed vertically) which showed greatest reactivity are plotted against A_{405} . The putative minimum epitope sequence is shown in bold and underlined.

the P1.10 VR family, 91 peptides of eight amino acids in length, overlapping by seven amino acids, were synthesized. The last two amino acids (VP) of the P1.10 VR were omitted from the synthesis schedule as these occur in all variants and it was assumed that they did not contribute to the minimum epitope. The peptide-bearing pins were reacted in ELISA with mAb MN20F4.17 (Fig. 3). The ELISA profile showed that most reactivity with the anti-P1.10 antibody occurred with the peptide of sequence KQNQRPTL and comparison with the other peptides showed that the sequence QNQRPTL was sufficient for binding of mAb MN20F4.17.

Binding of mAb MN20F4.17 is inhibited by a synthetic P1.10 peptide

The reactivity of mAb MN20F4.17 with a synthetic 14-amino-acid peptide was evaluated. The sequence of this peptide, HFVQNKQNQRPTLV, corresponded to the prototype P1.10 VR but lacked the terminal proline. The peptide did not react in ELISA when dried on to plates or in immunoblots when dot-blotted on to nitrocellulose filters, preventing a direct comparison of the binding of the peptide with whole cells of strain Z1040 to mAb MN20F4.17. Recognition of the peptide by mAb MN20F4.17 was demonstrated by using increasing amounts of the 14-amino-acid peptide with pure P1.10 antibody in a whole-cell competitive ELISA. The peptide inhibited mAb binding to whole cells at greater than 1 mg

Fig. 4. Competitive-binding ELISA between whole cells of the P1.10 VR strain and its synthetic VR peptide for mAb MN20F4.17. A synthetic peptide of amino acid sequence HFVQNKQNQRPTL, corresponding to the prototype P1.10 subtype, inhibited binding of mAb MN20F4.17 to whole cells of strain Z1040 expressing PorA containing the prototype P1.10 VR, whereas a negative control peptide of sequence CGPIKNKMSESPNKT (part of diphtheria toxin fragment DTB1) did not. Increasing concentration of each peptide is plotted against the mean absorbance readings from duplicate wells. ●, Dilutions of P1.10 peptide with 55 ng mAb per well; ○, dilutions of P1.10 peptide with 5.5 ng mAb per well; □, dilutions of negative control peptide with 55 ng mAb per well.

peptide per well (Fig. 4). The very high concentration of peptide required to out-compete the whole cells for binding to mAb suggested that the effect may be non-specific. However, a different synthetic 15-amino-acid peptide (part of diphtheria toxin fragment DTB1 with the amino acid sequence CGPIKNKMSESPNKT) did not inhibit binding of mAb MN20F4.17 to whole cells of strain Z1040 at concentrations up to 10 mg per well (Fig. 4).

Identification of variation in the P1.10 family by chain termination fingerprinting

The part of the *porA* gene encoding VR2 was examined by chain termination fingerprinting in the following clinical isolates: (i) 30 strains which had been shown to possess *porA* genes that encoded members of the P1.10 VR family by DNA hybridization (I. M. Feavers, A. J. Fox, S. Gray, D. M. Jones, J. Suker & M. C. J. Maiden, unpublished results) but which failed to react with mAb MN20F4.17 and (ii) 13 recent isolates of strains sero-subtyped by the Public Health Laboratory, Withington Hospital, Manchester as P1.10 using dot-blot with mAb

MN20F4.17. From the 30 strains that did not react with the mAb, 10 strains contained the prototype P1.10, 13 were P1.10a, four were P1.10c, one was P1.10d and two were P1.10f. Expression of PorA was not examined in these strains and therefore lack of expression remains an explanation of the failure of mAb MN20F4.17 to detect them. The 13 recent isolates of serosubtyped P1.10 strains all encoded a prototype P1.10 VR with one exception which was P1.10d.

DISCUSSION

There are two levels of antigenic variation of PorA: (i) distinct subtype families in both VR1 and VR2 and (ii) variation within these VR families. The biology of this variation has implications for the use of PorA as a target for epidemiological characterization and as a vaccine component. The application of mAb reagents was a major advance in the identification of PorA variants by serosubtyping (Abdillahi & Poolman, 1987, 1988a, b), and provided a degree of specificity and reproducibility that was difficult to achieve with polyclonal sera. However, molecular analyses of the *porA* gene have identified subtype variants not detected by the serosubtyping mAbs. The amino acid changes that distinguish members of PorA VR families result in different reactivities with serosubtyping mAbs (McGuinness *et al.*, 1991, 1993; Wedege *et al.*, 1993; Suker *et al.*, 1994). In some cases, these changes alter the minimum epitope sequence deduced from overlapping peptide experiments, a single amino acid change being sufficient to reduce or abolish antibody binding (McGuinness *et al.*, 1991). In other cases, the minimum linear epitope is still present in PorA proteins that do not bind the antibody, but the relative position of the peptide sequence has been altered by the insertion or deletion of amino acids (Wedege *et al.*, 1993). It has been proposed that the serosubtyping mAbs recognize a defined minimum peptide sequence as an epitope only when it is located at the apex of the putative loop formed by the VR (McGuinness *et al.*, 1993). Displacement of the minimum peptide sequence from the apex of the loop hence destroys the epitope.

This analysis of the reactivity of eight members of the P1.10 VR family illustrates the effects of naturally occurring PorA variant sequences on mAb recognition. The epitope recognized by the P1.10 serosubtyping mAb MN20F4.17 comprises the peptide sequence QNQRPTL and only PorA proteins with this sequence bind the antibody well. However, the following evidence suggests that the conformation in which the minimum epitope is presented also affects binding. The antibody can bind weakly to other variants which lack the minimum peptide sequence and this binding is abolished by boiling the variant proteins in SDS. A tethered peptide binds to the mAb well but the linear synthetic peptide in solution binds poorly. However, the fact that a linear peptide can bind to the antibody in solution means that further biochemical studies of the interaction of the antibody and peptide by Fab-peptide co-crystallization and NMR are possible.

These observations are inconsistent with the results of Brooks *et al.* (1995) who have reported other variants of P1.10 that do react with the serosubtyping mAb under routine assay conditions but do not contain the peptide sequence QNQRPTL. These variants, designated P1.10h, P1.10i and P1.10j are unusual in that they contain residues (tryptophan and alanine) that do not occur in the other P1.10 variants and are uncommon in all PorA VR2 loops. In addition, these variants did not occur in 301 serogroup A, B and C meningococci [233 strains from I. M. Feavers, A. J. Fox, S. Gray, D. M. Jones, J. Suker & M. C. J. Maiden, unpublished results; 55 strains in Suker *et al.* (1994) and 13 strains in the present study], examined in this laboratory. A limited range of subtype families in VR1 are found in combination with P1.10, the P1.5 family being most common. The P1.10 variant strains reported by Brooks *et al.* (1995) contain a variant of the P1.5 family (P1.5b) in five strains and a novel prototype VR1 (P1.29) in two strains which have not previously been reported in combination with P1.10 variants. It is possible that these peptide sequences alter the conformation of PorA enabling the variants P1.10h, P1.10i and P1.10j to bind to mAb MN20F4.17.

A comprehensive childhood vaccine against meningococci has yet to be developed (Frasch, 1985, 1989; Sanborn, 1987; Fine, 1991; Milagres *et al.*, 1994) and numerous protein antigens, including PorA, have been used as candidate vaccine components. Given the antigenic diversity of PorA, these vaccines must be multi-valent if they are to protect against different meningococcal strains. The composition of such vaccines depends on accurate strain characterization to determine the diversity of the target antigen. A successful vaccine also requires information on the human response to these components. Although serological typing may have a role in the elucidation of these factors, there are several problems associated with the design of vaccines on the basis of data obtained with murine mAbs. First, serosubtyping can underestimate the antigenic diversity of PorA because members of a VR family react unpredictably with a given serosubtyping mAb. Consequently, accurate interpretation of serosubtyping data is dependent on the definition of the binding properties of the serosubtyping mAbs and on the standardization and control of assay conditions. Secondly, the 'prototype' VR sequence, used to raise the antibody may not be representative of the subtypes found in the meningococcal population. For example, the P1.10a variant occurs more frequently than the prototype P1.10 in serogroup B and C strains. In addition, there is evidence that the epitopes identified by murine mAbs are not the same as the epitopes which are important in the human protective immune response (Delvig *et al.*, 1994). Finally, it is likely that the epitopes within the circulating meningococcal population may change over time due to immune selection following carriage in humans.

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