A linear B-cell epitope on the class 3 outer-membrane protein of *Neisseria meningitidis* recognized after vaccination with the Norwegian group B outer-membrane vesicle vaccine

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The class 3 outer-membrane protein (OMP) of *Neisseria meningitidis* is a potential target for bactericidal and opsonic antibodies in humans. Synthetic peptides spanning the class 3 OMP from the vaccine strain 44/76 (B:15:P1.7,16:L3,7) were synthesized on pins and screened with serum obtained from Norwegian adolescents immunized with a meningococcal serogroup B outer-membrane vesicle (OMV) vaccine. A strong IgG response to a single peptide (¹⁹FHQNGQVTEVT³⁰) located within loop 1 (VR1) was stimulated after three doses of OMV vaccine in three vaccinees selected on the basis of their antibody response to class 3 OMP. No clear linear B-cell epitopes were recognized by four different murine serotype 15-specific mAbs. A 23mer peptide (D63b2) containing loop 1 of the class 3 OMP was synthesized, and the IgG responses were measured in pre- and post-vaccination serum from 27 vaccinees. Specific IgG rose significantly in 37% of vaccinees 6 weeks after the second dose and in 74% of the vaccinees 6 weeks after the third dose of the OMV vaccine. Most immune sera reacted distinctly on immunoblots with denatured class 3 OMP, and the immunoblotting reactivity correlated strongly with concentration of the IgG antibodies specific for peptide D63b2. When added to a post-vaccination serum from one vaccinee, peptide D63b2 competed efficiently with the class 3 OMP for specific antibody binding on immunoblots and in pin ELISA. The results show that the significant part of the humoral response to the meningococcal class 3 OMP elicited by vaccination with the Norwegian OMV vaccine was directed against a single continuous epitope.

Keywords: *Neisseria meningitidis*, PorB protein, B-cell epitope, murine mAbs, synthetic peptides

INTRODUCTION

Neisseria meningitidis serogroup B strains are the primary cause of meningococcal infection in industrialized countries (Vedros, 1987). Elevated levels of serogroup B

disease have been registered in Norway since the mid-1970s, and an experimental outer-membrane vesicle (OMV) vaccine was used to immunize 90 000 adolescents. The estimated protection rate was 57% after 30 months of observation time in a double-blinded placebo-controlled protection trial (25 µg per dose of the vaccine administered twice at 6-week intervals) (Bjune *et al.*, 1991b). The vaccine was based on strain 44/76 (B:15:P1.7,16:L3,7) and contained the PorA protein (class 1), the PorB protein (class 3), the Opc protein and other components (Fredriksen *et al.*, 1991). Vaccination elicited antibodies to the P1.16 epitope of the PorA

Abbreviations: BCE, B-cell epitope; HLA, histocompatibility leukocyte antigens; mAb, monoclonal antibody; OMP, outer-membrane protein; OMV, outer-membrane vesicle; TCE, T-cell epitope; TFA, trifluoroacetic acid.

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protein, bactericidal IgG antibodies to the Opc protein (Rosenqvist *et al.*, 1993a, b; Wedege *et al.*, 1991) as well as opsonic antibodies that reacted with meningococci which express the class 3 outer-membrane protein (OMP) (Guttormsen *et al.*, 1993a). Details on the epitopes recognized by these various antibodies are lacking and their relevance to protection remains unknown.

Murine monoclonal antibodies (mAbs) to the class 2 and 3 OMP are the basis of the current serotyping scheme for serological subdivision of meningococci into about 20 serotypes (Frasch *et al.*, 1985). These mAbs are directed mainly to conformational epitopes (Zapata *et al.*, 1992) and have some protective activity in the infant rat infection model (Saukkonen *et al.*, 1987). Either class 2 or 3 major outer-membrane porins are expressed by all meningococcal strains, and they are encoded by two alleles of the *porB* gene (Hitchcock, 1989). The *porB* gene has been cloned and DNA sequences have been determined for the majority of serotype proteins (Feavers *et al.*, 1992; Ward *et al.*, 1992; Wolff & Stern, 1991; Butcher *et al.*, 1991; Zapata *et al.*, 1992). The class 3 OMP sequence has 80% homology with that of gonococcal protein IA and 60–70% homology with that of the class 2 OMP (Butcher *et al.*, 1991; Ward *et al.*, 1992). A molecular model for the PorB structure predicts eight surface-exposed hydrophilic loops of differing length (van der Ley *et al.*, 1991), with loops 1 and 5 bearing variable regions VR1 and VR2, respectively (Zapata *et al.*, 1992).

We have previously observed that the majority of post-vaccination human sera reacted with denatured class 3 protein on immunoblots (Wedege *et al.*, 1991), suggesting that they contain antibodies that recognize linear epitopes on the class 3 OMP. The aim of this study was to identify such linear BCE using synthetic peptides derived from the class 3 OMP. We also studied the kinetics of the IgG responses to these BCE in human serum. A small immunodominant region located within loop 1 of the class 3 OMP stimulated IgG antibodies in the majority of the vaccinees.

METHODS

OMV vaccine. Strain 44/76 (B:15:P1.7,16:L3,7) (Holten, 1979) was the ET-5 strain used for production of the serogroup B OMV vaccine. The vaccine consisted of deoxycholate-extracted OMVs adsorbed to aluminum hydroxide and contained the class 1–4 proteins, Opc protein, a class 5 protein which reacts with mAb P5.5, as well as small amounts of less well-characterized membrane proteins, plus about 8% lipooligosaccharide (Fredriksen *et al.*, 1991).

Human volunteers and serum samples. The following experiments were approved by the Ethical Committee for Medical Sciences in Norway. Twenty seven Norwegian adults were immunized at weeks 0 and 6, and after 4 years with 25 µg OMV meningococcal group B vaccine (produced by the National Institute of Public Health, Norway) (Bjune *et al.*, 1991a). Serum was drawn before immunization, 6 weeks after the first dose, 6 weeks and 4 years after the second dose, 6 weeks and 1 year after the third dose.

Murine mAbs. MN15A14H6 (serotype 15, IgG2a) (Abdillahi & Poolman, 1987; Abdillahi, 1988) was provided by Dr J. T.

Poolman, 2-2-P15 (serotype 15, IgG2a) (Zollinger *et al.*, 1984) was obtained from Dr W. D. Zollinger. Other serotype 15-specific mAbs, 188,C-1 (IgG3) and 152,D-8 (IgG1) (see below), were produced by the National Institute of Public Health, Norway.

Production of murine serotype 15-specific mAbs. Balb/C mice were immunized subcutaneously with 50 µg OMV from strain 44/76 emulsified in Freund's complete adjuvant at 0 and 2 weeks. Three days before fusion, mice were boosted intraperitoneally with 50 µg OMV diluted in PBS. Fusion of spleen cells with non-secretor murine myeloma cells was performed in the presence of polyethylene glycol 1450 (Kodak) by standard methods. The hybridoma supernatants were screened in ELISA for reactivity against OMV prepared from strain 44/76 as described (Harthug *et al.*, 1986). Positive hybridomas were expanded and culture supernatants were analysed by immunoblotting using OMV in the presence or the absence of 0.25% Empigen BB (Albright & Wilson) as described by Wedege *et al.* (1988). Class 3 OMP-specific hybridomas were cloned by limiting dilution, expanded and used as culture supernatants. Kit no. 93-6550 from Zymed was used for isotyping. The serotype 15 specificity of 152,D-8 and 188,C-1 mAbs was confirmed by whole-cell ELISA as described (Abdillahi & Poolman, 1987).

DNA sequence analysis. Chromosomal DNA was isolated from strain 44/76 as described (Kapperud *et al.*, 1990), except for an additional 2 h treatment of the DNA solution with boiled RNase A (20 µg ml⁻¹, Sigma) before the second phenol/chloroform extraction. DNA concentration and purity were determined spectrophotometrically and electrophoretically. The primers (synthesized by the Biotechnology Centre, Oslo) corresponded to the anti-sense strand of the region encoding the seven C-terminal amino acids of the class 3 protein plus the TAA stop codon (Maiden *et al.*, 1991) (P-733: 5'-TTA GAA TTT GTG GCG CAA ACC GAC-3') and encoded the first eight amino acids of the signal peptide (P-907: 5'-ATG AAA AAA TCC CTG ATT GCC CTG AC-3').

The PCRs were performed as described (Wedege *et al.*, 1993) except that the annealing temperature was 60 °C. PCR products were separated from the primers by centrifugation through a Chroma Spin + TE 100 column (Clontech). Sequencing primers (the two primers used in the PCR reaction plus the primers P-1472, 5'-TCC GTA CGC TAC GA(TC) TCT CCC G-3', and P-1473, 5'-CCG GCG TGG TAA GA-3') were labelled with [³²P]ATP, and the amplified material was sequenced by the Taq DNA sequencing kit (Perkin Elmer Cetus), followed by addition of formamide stop buffer. Products of the sequencing reactions were analysed with 6% (w/v) polyacrylamide sequencing gels and autoradiographed as described by Wedege *et al.* (1993).

Immunoblotting. Boiled OMV from strain 44/76 was used as antigen. After electrotransfer, blots were incubated with human sera (diluted 1:200) in the presence or the absence of 0.15% Empigen BB to enhance renaturation of the antigens (Wedege *et al.*, 1988). The intensity of the IgG antibody binding to the class 3 OMP was scored visually from 0 (no binding) to 4 (strong binding).

Synthetic peptides

(i) Solid phase peptide synthesis. The deduced amino acid sequence of the serotype 15 protein was used to manually synthesize multiple synthetic N-terminally acetylated 12mer peptides on pins which spanned the entire class 3 molecule with overlaps of 6 amino acids. Peptide synthesis was performed in duplicate using a commercially available Epitope scanning Kit

V2.0 (Cambridge Research Biochemicals) according to the manufacturer's instructions.

Other synthetic peptides (Table 1) were synthesized at 0.25 mmol scale using FastMoc technology with an automated peptide synthesizer (model 431A, Applied Biosystems) in one synthesis run. The peptides were synthesized using Rink resin (TentaGel S RAM, Rapp Polymere), which results in a C-terminal amide cap, and peptide D63a2 was acetylated at the N-terminus with acetic anhydride. Activation and coupling was done in the presence of 2-(1 H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate/diisopropylethylamine according to the manufacturer's instructions (FastMoc). Synthesis used Fmoc protected amino acids with trityl-(Gln, Asn), *t*-butyl-(Ser, Thr) or *t*-butyloxycarbonyl-(Lys) side-chain protection. The efficiency of the Fmoc de-protection of the growing peptide chain was monitored by following formation of a piperidine adduct at 301 nm.

Side-chain deprotection and cleavage from the resin support (6 h at room temperature) was achieved with 2.5% (v/v) 1,2-ethanedithiol, 2.5% water and 95% trifluoroacetic acid (TFA) (peptides D63a1 and D63a2) or with 7.5% (v/v) crystalline phenol, 2.5% 1,2-ethanedithiol, 5% thioanisole, 5% water, 80% TFA (peptide D63b2).

Peptides were recovered by precipitation with *t*-butyl methyl ether (Merck) followed by centrifugation. After lyophilization, peptides D63a1, D63a2 and D63b2 were dissolved in TFA, precipitated by dilution in 20 vols 1% acetic acid, 10% methanol, centrifuged, then redissolved in 50% methanol and lyophilized. This material was then dissolved in 1% acetic acid (D63a1), 5% acetic acid, 5% acetonitrile (D63a2), 1% acetic acid, 3% acetonitrile (D63b2) (Table 1), and purified by reversed phase-HPLC chromatography (Pep-S, C2/C18, 100 Å pore size, 12 µm × 22.5 mm × 25 cm, Pharmacia) using a gradient of acetonitrile, 0.1% TFA. Lyophilized peptides were stored at 20 °C.

(ii) Epitope mapping. The pins containing synthetic peptides were screened by ELISA for reactivity with human serum (diluted 1:500) or murine mAbs (152,D-8 and 188,C-1 mAbs were used as culture supernatants diluted 1:5; ascites fluids MN15A14H6 and 2-2-P15 were diluted 1:8000 and 1:5000, respectively). After incubation for 18 h at 4 °C, the pins were incubated with horseradish peroxidase conjugated rabbit anti-mouse Ig (1:1000) or rabbit anti-human Ig (γ -chains) (1:500) (Dakopatts) for 90 min as described (Virji & Heckels, 1989). Incubation of the pins with these conjugates alone resulted in no significant colour development. Each antibody sample was tested independently with duplicate sets of the pins.

(iii) Synthetic peptide ELISA. The optimal concentration of peptide D63b2 (5 µg ml⁻¹) and anti-human IgG (3 µg ml⁻¹) was determined by checkerboard titration. To quantify the peptide-specific IgG concentration in human sera, peptide D63b2 was coated on six rows of microtitre plates (MaxiSorp Nunc-Immuno plate F96, Nunc) in 0.05 M carbonate buffer, pH 9.6 at 35 °C until dry (at least 16 h). A calibration ELISA was set up using the remaining two rows of the plate which were coated with goat anti-human IgG (γ -chain specific) in 0.05 M carbonate buffer, pH 9.6 at 4 °C for 16 h. The plates were blocked (blocking buffer: PBS containing 2% (w/v) BSA and 0.05% Tween 20) for 90 min at room temperature. Tests with human serum (diluted 1:50 in blocking buffer) were performed in duplicate against both D63b2 peptide and uncoated plastic. Simultaneously, a dilution series of purified human IgG (Dako) was applied in the rows coated with goat anti-human IgG. After 90 min incubation at room temperature and washing, alkaline

phosphatase conjugate (diluted 1:500) of swine anti-human IgG (γ -chain specific) (Orion Diagnostica) was reacted for an additional 90 min. After washing in PBS containing 0.05% Tween 20, reactions were developed with *p*-nitrophenyl phosphate (1 mg ml⁻¹ in diethanolamine buffer) for 60 min at 36 °C, and read at 405 nm. Since the slopes of titration curves obtained in the peptide ELISA and in the anti-human IgG ELISA were similar, the concentration of IgG antibodies against peptide D63b2 was calculated according to the four parameters log-logistic model for single analytes (Plikaytis *et al.*, 1991) using the A_{405} data with the dilution series of the purified human IgG as a standard. Pearson product-moment correlations were computed between the peptide-specific IgG and immunoblotting scores (significance level $P \leq 0.05$).

(iv) Competition experiments. Reactivity with pins, on immunoblots and in the peptide ELISA were compared for the post-vaccination serum obtained 6 weeks after the third dose from vaccinee no. 1085 in the absence and the presence (20 µg per 40 µl serum) of peptide D63b2.

RESULTS

Analyses of linear epitopes recognized by human serum

The deduced amino acid sequence of the serotype 15 protein from strain 44/76 was used for synthesizing peptides on pins with maximal conservation of the linear structure of the variable regions (12mers overlapping by six amino acids).

Epitope mapping was done by selecting three vaccinees out of 27 volunteers (1061, 1467 and 1085) whose serum, taken after the third vaccine dose, contained IgG antibodies which showed strong binding to the class 3 OMP on immunoblots in the absence of detergent (Fig. 1, tracks 2, 4 and 6). These post-vaccination sera bound with

Fig. 1. Reactivity of human pre- and post-vaccination sera on immunoblots in the absence of detergent against the OMP preparation from strain 44/76. Tracks: 1 (pre-vaccination sera from vaccinee 1061); 2 (1061 post-); 3 (1467 pre-); 4 (1467 post-); 5 (1085 pre-); 6 (1085 post-); 7 (1085 post- in the presence of peptide D63b2; competition experiment). Arrowheads denote the position of the class 3 protein.

Fig. 2. ELISA reactivity of paired pre- and post-vaccination sera from two vaccinees with synthetic peptides on pins. Pre-vaccination serum from vaccinees 1061 and 1467 is shown in panels (a) and (c), respectively, and the corresponding post-vaccination serum, obtained after the third dose, is shown in panels (b) and (d). Synthetic peptides corresponding to the putative surface-exposed regions named loops 1 through 8 (Zapata *et al.*, 1992) are underlined. Arrows point to the peptide recognized by post-vaccination sera (¹⁹FHQNGQVTEVTT³⁰, peptide number 4). Results summarize two separate experiments on two different sets of pins. The bars represent the mean, and the error bars show SD of the mean.

different intensities to the class 3 OMP, in accordance with the quantitative reactivity of those sera with synthetic peptide D63b2 (see below). Binding was negligible in the pre-vaccination serum from these volunteers.

Pre- and post-vaccination serum from those three vaccinees were tested with the pins containing the synthetic 12mers peptides. The post-vaccination sera from all three vaccinees showed a significant increase in binding, relative to the pre-immune sera, with one 12mer peptide (¹⁹FHQNGQVTEVTT³⁰, peptide number 4) from the VR1 (loop 1) region (Figs 2 and 3). Strong binding to other peptides located on loops 2 and 4

Fig. 3. ELISA reactivity of (a) pre- and (b) post-vaccination sera from vaccinee 1085 with synthetic peptides on pins. (c) Post-vaccination serum in the presence of peptide D63b2 (competition experiment). Other details as in the legend to Fig. 2

(peptides 12 and 26, respectively) and on the trans-membrane regions following loops 2, 4 and 6 (peptides 14–15, 27–28 and 40, respectively), was comparable between the paired pre- and post-vaccination sera studied.

Mapping of the serotype 15 epitopes by murine mAbs

To locate regions important for serotype specificity, four murine serotype 15-specific mAbs were tested with the pin-bound 12mer peptides. Those mAbs bound to numerous unrelated peptides and showed no clear pattern of reactivity with a single epitope (Fig. 4). The strongest reaction for mAb 152,D-8 was with the peptide ¹⁹FHQNGQVTEVTT³⁰ (peptide number 4; also recognized by the post-vaccination human sera). Other mAbs (MN15A14H6, 2-2-P15 and 188,C-1) reacted with various peptides located within loops 2 and 4 or in the trans-

Fig. 4. ELISA reactivity of murine mAbs specific for serotype 15 protein with synthetic peptides on pins. mAbs: (a) 152,D-8; (b) MN15A14H6; (c) 2-2-P15; (d) 188,C-1. Other details as in the legend to Fig. 2.

membrane regions after loops 3 and 6 (also recognized by the pre- and post-vaccination human sera). All mAbs bound to the class 3 protein on immunoblots only in the presence of detergent (data not shown), suggesting that

murine serotype 15-specific mAbs recognize discontinuous epitopes (Zapata *et al.*, 1992).

Kinetics of the human IgG response

Peptides spanning different portions of loop 1 of the serotype 15 protein were synthesized and tested with human serum (Table 1). Since a 16mer peptide with capped N- and C-termini (D63a2) was recognized with about 10-fold lower efficiency (data not shown), compared to a 23mer peptide (D63b2) mimicking the entire loop 1 of the PorB protein, the latter was used to quantify IgG antibodies reacting with the linear epitope ¹⁹FHQNGQVTEVTT³⁰ in pre- and post-vaccination serum. Serum from vaccinees 1061, 1467 and 1085 obtained 6 weeks after the third dose contained 1.7, 5.9 and 8.2 µg IgG ml⁻¹, respectively, in quantitative agreement with the reactivity against the pin-bound epitope and with the intensity of reactivity with the class 3 OMP on immunoblots (Fig. 1, tracks 2, 4 and 6).

Additional sera (total of 161 sera from 27 participants of the vaccine trial) were also quantitated in peptide ELISA with D63b2 (Fig. 5). None of the vaccinees responded to the D63b2 peptide after the first vaccine dose. Six weeks after the second dose, significant rises (range 0.2–2.0 $\mu\text{g ml}^{-1}$) were observed in 10/27 (37%) of the vaccinees. Still stronger reactions were observed in sera taken 6 weeks after the third dose, when 13/27 of the vaccinees showed strong responses (range 1.0–8.8 $\mu\text{g ml}^{-1}$) and 7/27 weaker responses (range 0.3–0.9 $\mu\text{g ml}^{-1}$) in reactive IgG antibodies. All vaccinees exhibited a strong drop in IgG reacting with peptide D63b2 over the next 12 months, although the mean IgG concentration still remained approximately twofold higher than after the second dose (Fig. 5).

For the 20 vaccinees with detectable antibodies, a significant correlation ($r = 0.82$, $p < 0.05$) was found between the IgG specific for peptide D63b2 and the intensity of IgG binding to the class 3 OMP on immunoblots in the absence of detergent. Serum from one exceptional vaccinee (1510) bound to the class 3 OMP on immunoblots only in the presence of detergent (data not

Table 1. Synthetic peptides mimicking different portions of loop 1 of the serotype 15 protein

D number	Peptide	Sequence	Reactivity with human serum
D63a1	16mer	¹⁹ FHQNGQVTEVTTATGI ³⁴ -Am*	—
D63a2	16mer	†N-ac- ¹⁹ FHQNGQVTEVTTATGI ³⁴ -Am*	+
D63b2	23mer	¹² VETSRSVFHQNGQVTEVTTATGI ³⁴ -Am*	+++

*The C-terminus of each synthetic peptide was protected by an amide group (Am).

† The N-terminus of peptide D63a2 was protected by acetyl group (N-ac).

Fig. 5. Kinetics of synthesis following vaccination of IgG antibodies reacting with peptide D63b2 and with the class 3 OMP. ■, Concentration of IgG measured in ELISA with peptide D63b2; ○, immunoblotting scoring on an arbitrary scale from 0 to 3. Arrows indicate individual vaccinations at weeks 0, 6 and after 4 years. Data are the mean values for 27 vaccinees and the error bars indicate the standard error of the means.

shown) and showed inefficient binding ($0.4 \mu\text{g ml}^{-1}$) to peptide D63b2. Serum from six of the seven vaccinees with no detectable IgG antibodies to peptide D63b2 did react with the class 3 OMP band on immunoblots, suggesting that that protein does contain additional epitopes not identified here.

No reaction was detected with the 16mer peptide D63a1, which differs from D63a2 in having a charged N-terminus, suggesting that charged amino acids near the human BCE hamper interactions with reactive antibodies (Delvig *et al.*, 1994).

Competition experiments

The 23mer peptide D63b2 was tested for competitive binding using the post-vaccination serum from a vaccinee with a high level of IgG antibodies to that peptide ($1085, 8.2 \mu\text{g ml}^{-1}$). The presence of $20 \mu\text{g}$ peptide D63b2 in $40 \mu\text{l}$ serum resulted in a distinct reduction in the intensity of binding to the class 3 band on immunoblots (Fig. 1, track 7). We also observed a strong reduction (about 80%) in the IgG-reactive with D63b2-coated on ELISA plates as well as in the reaction with the 12mer peptide $^{19}\text{FHQNGQVTEVT}^{30}$ on pins (Fig. 3c). These data suggest that the BCE located on loop 1 of the class 3 OMP is the immunodominant linear epitope.

DISCUSSION

Several authors have reported immunodominant B-cell epitopes recognized by murine mAbs on different proteins expressed by *N. meningitidis* (McGuinness *et al.*, 1993, 1990; Morelli *et al.*, 1994; Hobbs *et al.*, 1994; Abdillahi & Poolman, 1987; Abdillahi, 1988; Zollinger *et al.*, 1984; Thompson *et al.*, 1993; Tinsley *et al.*, 1992; Virji *et al.*,

1989). T-cell epitopes recognized by human peripheral blood mononuclear cells have also been described (Wiertz *et al.*, 1992), but data on human BCE in meningococcal proteins are more limited (Delvig *et al.*, 1994; Morelli *et al.*, 1994; de Cossio *et al.*, 1992), partially due to the discontinuous nature of many epitopes recognized by human antibodies (Laver *et al.*, 1990).

Using pin-bound 12mer synthetic peptides overlapping by 6 amino acids, we identified an immunodominant continuous epitope ($^{19}\text{FHQNGQVTEVT}^{30}$) located on loop 1 of the serotype 15 protein which was recognized after vaccination with the Norwegian OMV vaccine. Recently, the total antibody responses against the PorB protein have been quantified using purified class 3 protein in serum from patients with meningococcal disease (Guttormsen *et al.*, 1993b). Apart from a significant increase in specific IgG antibodies in 22/25 of those patients, the study also showed considerable heterogeneity in IgG concentrations and subclasses as well as in the kinetics of the IgG responses. We used a 23mer synthetic peptide (D63b2) covering loop 1 of the serotype 15 protein to quantify the IgG responses in 161 pre- and post-vaccination sera from 27 vaccinees, and also observed a similar heterogeneity of the IgG responses to this region. A significant IgG response to the D63b2 peptide was determined in 37% of vaccinees after the second vaccine dose, but a consistent immune response was first found in 74% of vaccinees after the third dose. The mechanism of this effect is not understood, but may involve inefficient processing and/or presentation of immunogenic peptides, derived from the class 3 OMP, by antigen-presenting cells.

The following observations demonstrate that the overall immune response against the serotype 15 protein, elicited by vaccination, is directed against both linear and conformational epitopes. The pre- and post-vaccination sera bound to other 12mer peptides (covering loops 2 and 4, and the trans-membrane regions following loops 2, 4 and 6) (Figs 2 and 3), also recognized by at least three different murine serotype 15-specific mAbs (MN15A14H6, 2-2-P15 and 188,C-1) (Fig. 4), thus indicating other parts of the PorB protein involved in the putative discontinuous epitope. In addition, seven vaccinees failed to develop any detectable response to the D63b2 peptide after three immunizations, although six of those responded against the class 3 OMP on immunoblots in the presence of detergent, probably recognizing conformational epitopes which would not be detected by pin ELISA tests. Taken together, these observations suggested that, in addition to the linear BCE, undefined discontinuous epitopes on the class 3 OMP are involved in the human immune response to the OMV vaccine.

Recent advances in peptide synthesis make the B-cell epitope described here potentially useful in the development of a synthetic meningococcal vaccine. To this end, synthetic peptides covering the BCE may be used as cyclic peptides or in combination with T-cell epitopes as a multiple antigen peptides (MAP) system, which were shown to stimulate production of bactericidal antibodies

in mice (Christodoulides *et al.*, 1993; Christodoulides & Heckels, 1994). Alternatively, different synthetic peptides encompassing the potentially protective meningococcal B-cell epitopes and T-cell epitopes can be combined within one construct using template-assembled synthetic proteins design (Mutter & Vuilleumier, 1989).

To generate antibody responses to protein antigens, immunogenic peptides, released in endocytic vesicles during the processing of a given protein, must associate with the highly polymorphic HLA class II glycoproteins. The specificity of this interaction is determined by polymorphic amino acid residues located in the peptide-binding groove of the HLA class II molecule (reviewed by Vignali & Strominger, 1994, and by Chicz & Urban, 1994). Consequently, the observed heterogeneity in immune responses to the 23mer D63b2 might reflect the HLA class II restriction. Indeed, analysis of the putative HLA class II structural motifs (Chicz *et al.*, 1992; Matsushita *et al.*, 1994) enabled us to recognize an HLA-DR1 binding motif (¹⁶R----Q---V²⁵) which partially overlaps with the human BCE on the class 3 OMP, consisting of 10 amino acids of which the first is positively charged, the sixth is a hydrogen-bond donor and the tenth is hydrophobic. The frequency of HLA-DR1 in the Norwegian population is about 0.09 (Spurkland *et al.*, 1992), and it is unlikely that this motif alone could account for the high frequency of responses to peptide D63b2. No DR4-binding motifs (DRB1*0405 or DRB1*0406) were found.

The results indicate that the Norwegian serogroup B meningococcal OMV vaccine is efficient in stimulating production of antibodies specific for a human linear BCE located within loop 1 of the serotype 15 protein. The relevance of such antibodies for protection is currently under study.

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