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# T-Cell Responses to Outer Membrane Proteins of *Neisseria meningitidis*: Comparative Study of the Opa, Opc, and PorA Proteins

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**Former studies have shown that the class 5 outer membrane proteins (Opa and Opc proteins) of *Neisseria meningitidis* are at least as immunogenic as meningococcal porin proteins. High antibody titers to class 5 proteins have been observed in sera obtained during convalescence after meningococcal infection. A strong increase in anti-class 5 antibodies has also been observed in vaccinees who received a meningococcal outer membrane vesicle preparation. The enhanced B-cell response to class 5 proteins may be due to the presence of immunodominant helper T-cell epitopes in these proteins. In order to investigate this hypothesis, we tested purified Opa, Opc, and class 1 proteins for recognition by human T cells. A hierarchy of T-cell immunogenicity was observed among the outer membrane proteins, the Opa protein being more immunogenic than the other proteins. In most cases, the proliferative responses elicited by Opc were higher than the responses observed for the class 1 protein. The epitopes recognized by the immune T cells were identified by using overlapping synthetic peptides spanning the protein sequences of OpaB, Opa5d, and Opc.**

The major outer membrane proteins (OMPs) of *Neisseria meningitidis* have been divided into five classes based on molecular weight and proteolytic peptides (25). The porin proteins, class 1 (PorA) and class 2/3, are stably expressed by virtually all meningococcal isolates, although a certain antigenic heterogeneity has been demonstrated (1, 5, 8, 11, 22, 29). In addition, each meningococcal strain can contain three or four *opa* genes encoding distinct Opa proteins and one *opc* gene encoding the Opc protein, which together are referred to as class 5 proteins (4, 9, 14). Opc (formerly 5C) proteins differ from Opa in genetic regulation (19) and biochemical and immunological properties (2, 14). While variable regions of Opa proteins result in size differences and immunological heterogeneity (4, 9), the Opc protein is of constant size and antigenicity (2, 14, 19).

The sequences of the Opa proteins can differ dramatically among unrelated meningococcal strains, especially in the semi- and hypervariable regions called SVR, HVR1, and HVR2 (4, 9). In addition, expression of these proteins is hypervariable, and any one strain can express between none and all four of the class 5 proteins for which it possesses genes (2, 3, 34). Because of this high degree of variability, the clinical relevance of antibodies directed to class 5 OMPs is expected to be limited, whereas the more limited variability of class 1 proteins indicates that bactericidal antibodies (20, 30, 31) to these proteins may be more useful for protection against meningococcal disease. However, convalescent-phase sera reacted more strongly with class 5 proteins than with class 1, 2, or 3 proteins (16), and a strong increase in antibodies to class 5 proteins was also observed in vaccinees who received a meningococcal outer membrane vesicle preparation (12, 18, 30).

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Individual class 5 proteins are not more surface exposed than other membrane proteins and do not contain excessive surface-exposed regions compared with other membrane proteins. One possible explanation of the relative immunodominance of class 5 OMPs might be based on the fact that antibody production by B cells is regulated by helper T cells which recognize specific T-cell epitopes. Isotype switch, level of immunoglobulin (Ig) synthesis, Ig affinity maturation and the induction of a secondary response are T-cell-dependent phenomena. Hence, the enhanced B-cell response towards the class 5 proteins may be due to the presence of immunodominant T-cell epitopes effectively activating helper T cells.

In order to investigate the relative immunodominance of class 5 proteins, we tested purified Opa and Opc proteins as well as synthetic peptides derived from class 5 protein sequences for recognition by T cells obtained from the peripheral blood of human volunteers. Previously, this approach has been applied successfully to identify T-cell epitopes occurring in the meningococcal class 1 OMP (32).

## MATERIALS AND METHODS

**Volunteers.** A group of 18 healthy adults (29 to 55 years old) with negative anamnesis results for meningococcal disease participated in this study. The volunteers were not selected on a particular basis. Peripheral blood mononucleated cells (PBMC) obtained from these individuals demonstrated a positive proliferative response to one or more of the purified OMPs (see below). The high frequency of donors with positive T-cell responses to the OMPs in a normal population is likely to be caused by repeated immunization by asymptomatic infections. This explanation is supported by the fact that antibodies directed against various meningococcal antigens (Ags) such as OMPs and lipopolysaccharide (LPS) were present in the serum of all volunteers.

Human leukocyte antigen (HLA) typing of the volunteers was performed by the standard National Institutes of Health lymphocytotoxicity test for HLA-ABC and by the propidium iodide method for HLA-DR and DQ, using well defined sets of alloantisera and monoclonal antibodies (MAbs) (33).

**Peptide synthesis.** The peptides used in this study were assembled with an AMS 422 simultaneous multiple peptide synthesizer equipped with a 48-column reaction block (ABIMED Analysen-Technik, Langenfeld, Germany) as described earlier (32) except that Fmoc-Trp(Boc)-OH ($N^{\alpha}$-fluorenylmethoxy-

carbonyl-$N^{in}$-*tert*-butyloxycarbonyl-L-tryptophan) was used instead of Fmoc-Trp-OH ($N^{\alpha}$-fluorenylmethoxycarbonyl-L-tryptophan). Peptides with N-acetylated N termini and amidated C termini (uncharged) were prepared at the 10-μmol scale. Three series of 18-mer peptides overlapping by 12 amino acids were assembled, spanning the OpaB (9), Opa5d (4), and Opc (GenBank M80195) protein sequences. The peptides belonging to a particular protein were assembled simultaneously. The purity of all peptides was determined by reverse-phase high-pressure liquid chromatography (HPLC) analysis and was generally found to be 85 to 95% and occasionally lower (50 to 85%). The crude peptides were used in immunological experiments. To obtain stock solutions, the peptides were dissolved in 200 μl of 6 M urea–Tris (pH 7.0) and diluted with culture medium (32) to a concentration of 2 mM. In the immunological experiments, a final peptide concentration of 6 μM was used.

**OMPs.** The Opc (GenBank M80195), OpaB (GenBank U03406), and OpaF proteins were purified from meningococcal outer membranes as described previously (2). Opa5d (GenBank X63110) was originally isolated from strain FAM18, a serogroup C ET-37 complex bacterium (4). The class 1 OMP was purified (17) from strain HIII-5, a mutant of reference strain H44/76 (B:15:P1.7,16), lacking the class 2/3 OMP (24). To obtain stock solutions, the proteins were dissolved in 1 ml of 6 M urea–Tris (pH 7.0). Prior to the experiments, the proteins were diluted with culture medium to a final concentration of 0.25 μg per well.

**Proliferation assays.** Freshly isolated PBMC ($10^5$ per well) were cultured in the absence or presence of antigen in 96-well round-bottomed microculture plates (final volume, 150 μl per well) as described before (32). After 5 days, the cultures were pulsed with 0.5 μCi of [$^3$H]thymidine. Sixteen to 18 h later, all 96 wells were harvested simultaneously with a 96-well sample harvester (Pharmacia/LKB, Turku, Finland). The complete filters containing 96 spots were placed in plastic bags. Scintillation liquid (Pharmacia/LKB) was added, and the bags were sealed. Radioactivity incorporated into DNA was determined with a Pharmacia/LKB 1205 Betaplate counter. The results obtained with six replicate cultures are expressed as the stimulation index (SI), which was calculated as the ratio of counts per minute (cpm) obtained in the presence of Ag to the cpm obtained in the absence of Ag (medium alone).

TABLE 1. Proliferative response of PBMC to purified class 5 and class 1 OMPs[a]

| Donor | HLA phenotype | | Response to purified protein (SI) | | | |
|---|---|---|---|---|---|---|
| | DR | DQ | OpaB | OpaF | Opc | P1.7,16 |
| 1 | 4,10 | 5,8 | 3 | 2 | <1 | 3 |
| 2 | 12(5),13(6) | 6,7 | 8 | 8 | 5 | 2 |
| 3 | 3,7 | 2 | 20 | 23 | 30 | 7 |
| 4 | 15(2),13(6) | 6 | 23 | 10 | 20 | 2 |
| 5 | 11(5),7 | 2,7 | 15 | 15 | 5 | 9 |
| 6 | 11(5),10 | 5,7 | 33 | 28 | 5 | 7 |
| 7 | 16(2),11(5) | 5,7 | 4 | 16 | 5 | 2 |
| 8 | 15(2),4 | 1,8 | 13 | 18 | 6 | 3 |
| 9 | 15(2),9 | NT[b] | 16 | 13 | 10 | 1 |
| 10 | 15(2),8 | 4,6 | 18 | 14 | 6 | 4 |
| 11 | 15(2) | NT | 29 | 18 | 14 | 2 |
| 12 | 11(5) | 7 | 93 | 74 | 4 | 4 |
| 13 | 3,7 | 2 | 10 | 11 | 10 | 5 |
| 14 | 1,13(6) | 5,6 | 20 | 20 | 11 | 9 |
| 15 | 3,12(5) | 2,7 | 11 | 11 | 2 | 2 |
| 16 | 1,3 | 2,5 | 1 | 2 | 1 | <1 |
| 17 | 2,7 | NT | 5 | 7 | 14 | 4 |
| 18 | 1,7 | 5,9 | 45 | 23 | 6 | 38 |

[a] PBMC were incubated with the OMPs (0.25 μg per well) for 6 days. Proliferation was measured as indicated in Materials and Methods. Results of three replicate cultures are presented as the SI, which was calculated as (experimental cpm + Ag)/(control cpm − Ag). Background proliferation was 50 to 200 cpm for donors 4, 6, 7, 8, 9, 12, 14, 15, and 18, 200 to 800 cpm for donors 1, 3, 5, 10, 11, 13, and 17, and 800 to 3,000 cpm for donors 2 and 16.

[b] NT, not tested.

## RESULTS

**T-cell recognition of Opa, Opc, and class 1 proteins.** PBMC were obtained from 18 randomly selected healthy individuals whose sera possessed antibodies to purified class 1 OMP and a various number of class 5 proteins (Opc, OpaA, OpaB, OpaD, OpaF, or OpaG; data not shown). Such antibodies might reflect a response to intermittent asymptomatic infections (carriage). Proliferative T-cell responses to meningococcal OMPs were observed in most donors. This observation is in accordance with previous experiments performed in our laboratory. When lymphocytes from about 40 people were tested for T-cell recognition of meningococcal membrane proteins, a response to one or more OMPs was observed for most of the volunteers.

PBMC from most donors demonstrated higher proliferative responses to purified OpaB or OpaF proteins than to the Opc or P1.7,16 class 1 protein (Table 1). In many cases, similar proliferative responses were observed to both Opa variants. The responses recorded for the Opc protein were usually higher than those for the class 1 OMP. Average SIs detected for the OpaB, OpaF, Opc, and P1 proteins were 20, 17, 8.5, and 6, respectively. Using the Student *t* test for paired samples (with $P < 0.05$ being significant), the hypotheses OpaB > P1 ($P = 0.007$), OpaF > P1 ($P = 0.009$), OpaB > Opc ($P = 0.04$), and OpaF > Opc ($P = 0.046$) were significant. The hypothesis Opc > P1 ($P = 0.31$) has to be rejected.

**Identification of T-cell epitopes occurring in meningococcal class 5 proteins.** Figures 1, 2, and 3 show the amino acid sequences of the overlapping peptides derived from the DNA sequences encoding the OpaB (9), Opa5d (4), and Opc (14) proteins, respectively. The two Opa proteins demonstrate significant heterogeneity, particularly within the semivariable region and the two hypervariable regions (the differences are underlined in the Opa5d sequence shown in Fig. 2). The last C-terminal amino acid residues of the Opa proteins are identical, and the synthetic peptides corresponding to this part of the sequence (Opa5d peptides 32 to 35) were only prepared once.

Schematic diagrams of the proliferative responses obtained with PBMC from HLA-typed immune adults to the series of overlapping peptides spanning the OpaB, Opa5d, and Opc proteins are presented in Fig. 4. A positive response to one or more Opa and Opc peptides was recorded with PBMC from most donors, and virtually all peptides were recognized by PBMC from at least one donor. Peptides derived from OpaB and Opa5d that differed by only a single amino acid yielded significantly different responses in many cases. Unfortunately, Opa5d was not available in a purified form, and no data on T-cell recognition of this protein are available. No significant T-cell proliferation was seen to the Opc protein or to the peptides derived therefrom with PBMC from some volunteers (donors 1, 15, and 16).

A stacked bar diagram representing a compilation of the positive responses that were observed to each of the OpaB-derived peptides is shown in Fig. 5. It is interesting that many positive responses were recorded for peptides occurring outside the hypervariable regions of the Opa protein (e.g., peptides 6 to 12 and 24 to 29). Likewise, significant proliferation was observed to peptides corresponding to relatively conserved regions of the Opa5d protein (i.e., peptide 19 to 23 [Fig. 4, middle]).

Overlapping peptides (20-mers with 12 overlapping amino acid residues) spanning the meningococcal class 1 OMPs have been assembled and tested for T-cell recognition previously (32). In order to compare the immunogenicity of the various OMPs at the molecular level, the P1 peptides were tested in the current study as well (data obtained with PBMC from the individual donors are not shown). A summary of the peptide-specific T-cell responses is presented in Table 2. The number of positive responses ranged from 21 to 37% of the total number of tests performed. The percentage of positive responses with an SI > 10 was higher for the Opa peptides than for either the Opc or the class 1 OMP peptides.

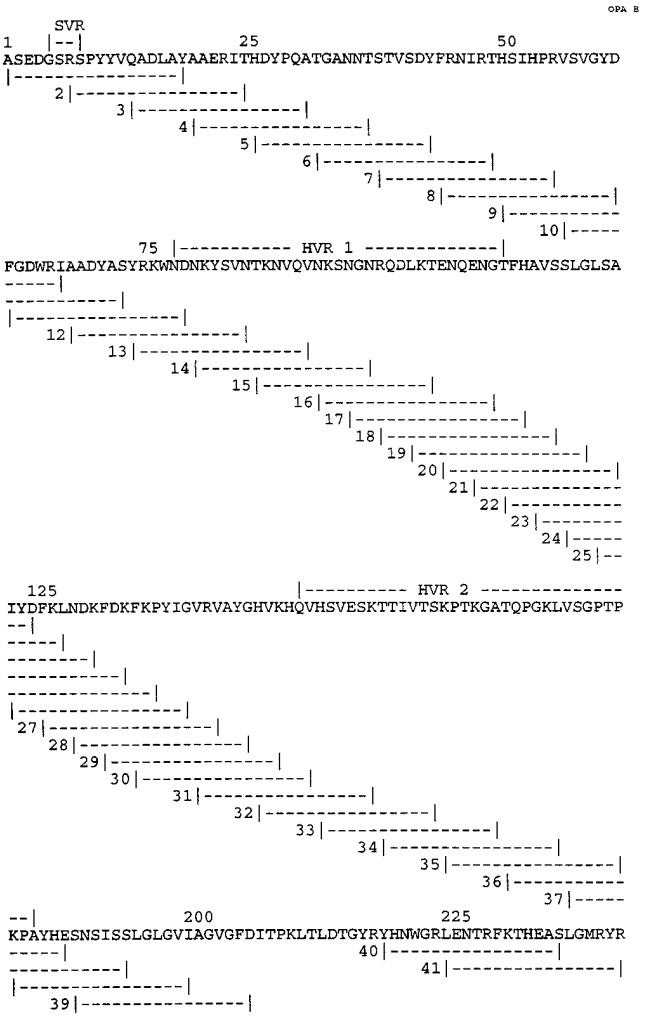


FIG. 1. Amino acid sequence of the OpaB class 5 protein of serogroup A, subgroup IV-1 of *N. meningitidis* (9). Synthetic 18-mer peptides overlapping by 12 residues are indicated with their code numbers. The 192 to 232 region occurs in the Opa5d protein as well. The synthetic peptides corresponding to this sequence were assembled only once and included in the Opa5d series (i.e., peptides Opa5d 32 to 35; see Fig. 2). The semivariable and hypervariable regions (SVR and HVR, respectively) are indicated above the protein sequence.

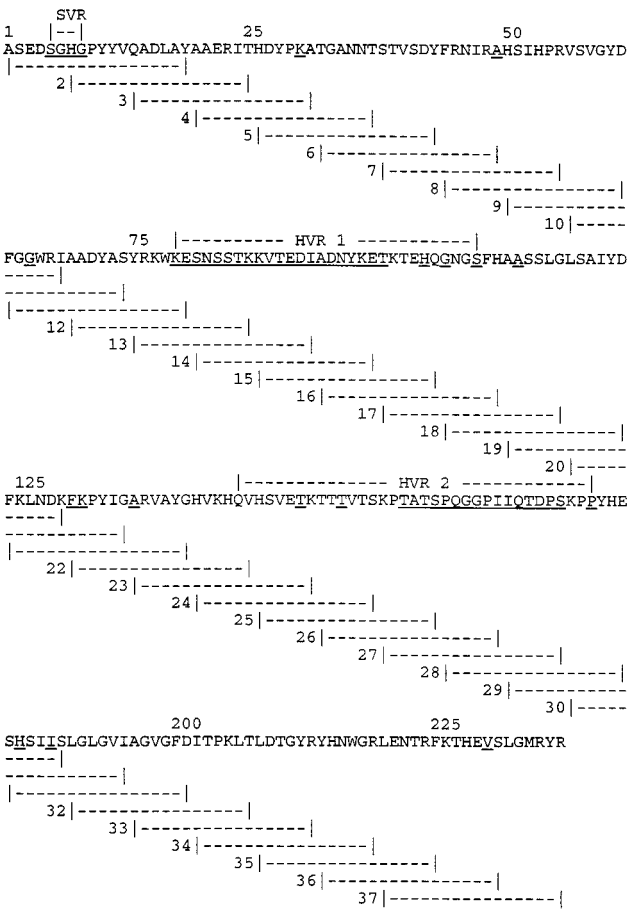


FIG. 2. Amino acid sequence of the Opa5d class 5 protein of serogroup C, ET-37 complex of *N. meningitidis* (4). The amino acid residues that differ between the Opa5d and OpaB proteins are underlined. See Fig. 1 legend for details.

## DISCUSSION

Purified meningococcal class 5 (Opa and Opc) proteins and a class 1 protein were tested for recognition by T cells isolated from the peripheral blood of 18 volunteers. Surprisingly, most of the donors demonstrated positive responses to the OMPs. The volunteers were healthy individuals with negative anamnesis results for clinical meningococcal disease. The high frequency of positive T-cell responses to the OMPs is likely to be caused by repeated immunization by asymptomatic infections. This explanation is supported by the fact that antibodies directed against various meningococcal antigens such as OMPs and LPS were present in the serum of these volunteers.

The comparative analysis of T-cell responses to meningococcal OMPs revealed an interesting hierarchy in T-cell immunogenicity, the Opa proteins being the most immunogenic, followed in most cases by the Opc and then the class 1 protein. T-cell recognition of meningococcal strain H44/76 class 3 OMP has been investigated in a previous study (33). Although higher protein concentrations were used in that study, the class 3 protein elicited much lower responses than the class 1 protein purified from that strain. Thus, the relative T-cell immunogenicity of the meningococcal OMPs observed so far can be summarized as follows: Opa $>$ Opc $\geq$ class 1 $>$ class 2/3. Although only few purified proteins have been tested, this order may apply to other Opa and class 1 proteins as well. Class 1 and Opa proteins from different strains demonstrate a high degree of sequence homology with the exception of two hypervariable regions (8, 9, 11, 22, 24). Interestingly, the dominant T-cell epitopes were predominantly found to occur in conserved regions of the Opa proteins (see below).

So far, no studies in which Opc and other class 5 proteins have been compared for immunogenicity have been published. However, convalescent-phase sera reacted more strongly with class 5 proteins than with class 1, 2, or 3 proteins (16), and a strong increase in antibodies to class 5 proteins was also observed in vaccinees who received a meningococcal outer membrane vesicle preparation (18, 30). The outcome of surface Ig-initiated B-cell activation is strongly dependent on the helper signals provided by major histocompatibility complex (MHC) class II-restricted $CD4^+$ T helper 2 cells. Hence, the significant T-cell immunodominance of the Opa proteins presents an attractive explanation for the high antibody titers to these proteins observed upon immunization or convalescence. It is unlikely that the relative prevalence of Opa-directed an-


FIG. 3. Amino acid sequence of the Opc protein of *N. meningitidis* (GenBank accession number M80195). Note that the C-terminal amino acids differ from the sequence in reference 14 for reasons explained in reference 19. The C-terminal phenylalanine was not included in the peptides synthesized.

tibodies is due to qualitative or quantitative dominance of the B-cell determinants present on these proteins. The class 1, class 2/3, and class 5 proteins all possess surface loops containing recognizable B-cell epitopes (2, 13, 14, 26). Gel electrophoresis of meningococcal outer membranes usually reveals expression of Opa proteins in amounts that are smaller than or equal to the amounts of class 1 and class 2/3 proteins. Additional experiments are required to support the conclusion that the high antibody titers to the Opa proteins are indeed due to T helper cells, predominantly activated by Opa-specific T cells. Verification of this hypothesis in vaccinees or reconvalescents represents an alternative. Furthermore, the analysis of cytokine profiles in supernatants of T lymphocytes, preferably T-cell clones, activated with the various meningococcal OMPs or peptides derived therefrom may provide evidence for the existence of immunomodulation of the B-cell response by Opa-specific T cells.

Specific Opa proteins may play a role in invasion of epithelial cells, whereas Opc is involved in adhesion and invasion of both epithelial and endothelial cells (27, 28). Although specific antibodies might prevent invasion (23, 27) as well as being bactericidal (18), antibodies stimulated by carriage are unlikely to be protective against disease caused by other meningococci because of the high degree of sequence variability of Opa proteins. Furthermore, all these proteins are subject to phase variation (3, 15, 23, 34), which enables the bacteria to avoid antibody binding. Thus, the contribution of such antibodies to protection against meningococcal disease would probably be limited.

The epitopes recognized by the class 5 protein-directed T cells were identified by using overlapping synthetic peptides spanning the sequences of the OpaB, Opa5d, and Opc proteins (Fig. 4). The responses of the donors appeared to be very diverse, which in part reflects the polymorphism of the HLA molecules involved in presentation of the peptides to the immune T cells. Furthermore, meningococcal immune status seemed to vary among the donors. There is not always a correlation between T-cell proliferation to a purified protein and overlapping synthetic peptides encompassing that protein. For example, in the case of OpaB, this phenomenon was clearly observed for cells from donors 5 and 14; significant responses to the purified protein were observed, whereas proliferation to the overlapping peptides was weak or absent, respectively. Failure in peptide synthesis is not likely to be responsible for these paradoxical results. The purity of all peptides was determined by reverse-phase HPLC analysis and was usually found to be 85 to 95%. For a number of peptides, the identity was confirmed by fast atom bombardment mass spectrometry. Furthermore, virtually all peptides elicited a response in lymphocytes from one or more other volunteers, which provides additional evidence for the capacity of the peptides to induce T-cell proliferation. Alternatively, it is unlikely that minute contaminations in the protein preparations are inducing the T-cell proliferation. The possibility of aspecifically induced polyclonal T-cell proliferation, such as can be elicited by T-cell mitogens or superantigens, is also not very likely, because lymphocytes from some individuals do not respond to a particular meningococcal protein. As an additional control for the antigen specificity of the T-cell responses, proliferation experiments were performed with lymphocytes isolated from cord blood. With these "naive" cell populations, no proliferation to the OMPs or any of the synthetic peptides used in this study was observed (results not presented).

Perhaps the mechanism explaining the paradox is related to a loss of T-cell epitopes sensu strictu. Although most peptides induce T-cell proliferation, a loss of certain T-cell epitopes that are present in the parental protein cannot be ruled out. A possible explanation for unresponsiveness to a particular T-cell epitope is an unfortunate choice of overlapping sequences of adjacent peptides. The overlapping sequence may not actually encompass the entire epitope, i.e., may lack one or more crucial residues. Also, the termini of a synthetic peptide may interfere with MHC and/or T-cell receptor interaction, thus prohibiting T-cell recognition of a sequence that would have been accessible if derived from the original protein by natural processing. Alternatively, proteolytic cleavage sites may be more easily accessible in the synthetic peptide than in the intact protein, leading to rapid degradation of the synthetic peptide.

A summary of the T-cell responses observed to the peptides is presented in Table 2. The results obtained with a series of overlapping synthetic peptides spanning the meningococcal class 1 OMP have been included in this overview. The proliferative responses observed to the P1-derived peptides in the current study were comparable to those observed previously (32) (data not shown). Since virtually all class 5 and class 1 OMP-derived peptides were recognized, it is unlikely that the dissimilar T-cell immunogenicity of the various proteins is due to the number of T-cell determinants occurring in the protein

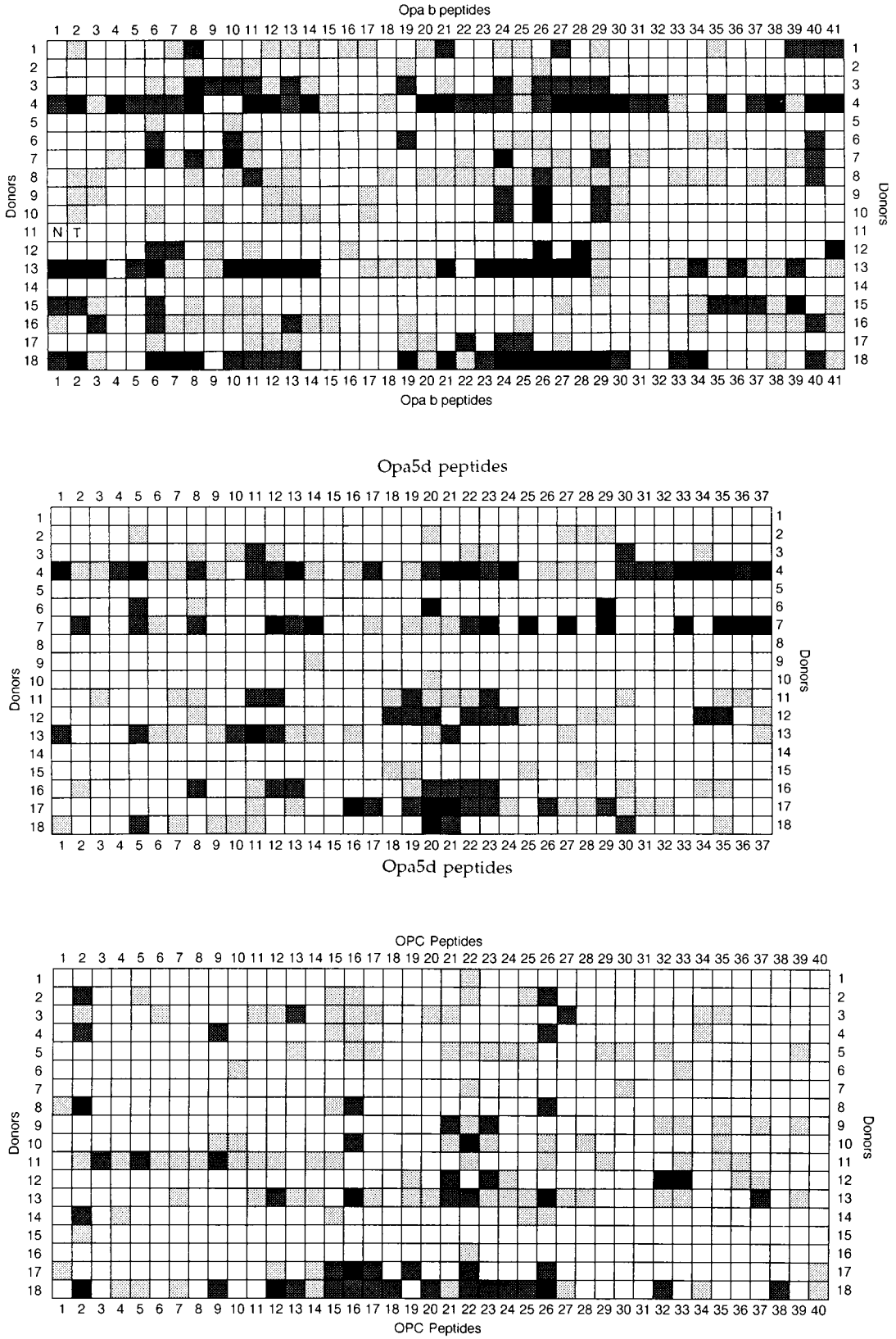


FIG. 4. (Top) Schematic diagram of the proliferative responses obtained with PBMC from HLA-typed immune adults to 41 overlapping peptides spanning the meningococcal OpaB protein (Fig. 1). The HLA-DR and DQ phenotypes of the donors are shown in Table 1. The results of six replicate cultures are presented as the SI, which was calculated as (experimental cpm + Ag)/(control cpm − Ag). Open boxes, SI $< 3$; lightly shaded boxes, SI $= 3$ to 5; darkly shaded boxes, SI $= 5$ to 10; solid boxes, SI $> 10$. Background proliferation was 50 to 200 cpm for donors 4, 6, 7, 8, 12, and 18, 200 to 800 cpm for donors 1, 3, 9, 10, 11, 13, and 17, and 800 to 3,000 cpm for donors 2, 5, 14, 15, and 16. PBMC from donor 11 were not tested with these peptides. (Middle) Diagram of the proliferative responses observed for 37 overlapping peptides spanning the meningococcal Opa5d protein (Fig. 2). Background proliferation was 50 to 200 cpm for donors 4, 6, and 12; 200 to 800 cpm for donors 3, 7, 9, 11, 13, 16, 17, and 18; and 800 to 3,000 cpm for donors 1, 2, 5, 8, 10, 14, and 15. (Bottom) Proliferative responses to 40 overlapping peptides spanning the Opc protein (Fig. 3). Background proliferation was 50 to 200 cpm for donors 4, 6, 7, 8, 9, 12, 14, 15, and 18; 200 to 800 cpm for donors 1, 3, 5, 10, 11, 13, and 17; and 800 to 3,000 cpm for donors 2 and 16.

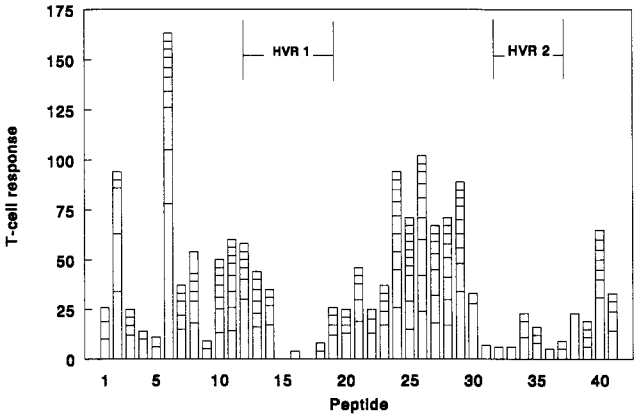


FIG. 5. Histogram representing the cumulative SI (SI > 3) to each of the overlapping peptides derived from the OpaB protein (17 immune volunteers). The individual responses are shown in Fig. 4 (top).

sequences. However, the magnitude of the proliferative responses observed appeared to be very high for certain Opa peptides. An SI of ≥10 occurred for 8% of the total number of tests performed with the Opa peptides, whereas only 1% of the tests with Opc peptides and 3% of the tests with P1 peptides elicited an SI of ≥10.

The enhanced T-cell proliferation observed for the purified Opa proteins and some of the peptides derived therefrom can be explained in several ways. Opa-specific T cells may be present in the peripheral blood at relatively high frequencies. To be recognized by helper T cells, antigenic determinants have to be presented by MHC class II molecules occurring on the antigen-presenting cells. Hence, the Opa-induced proliferative response per se may also be enhanced by efficient uptake, processing, and presentation by the antigen-presenting cells. The stability of the peptide-MHC interaction depends on the presence of proper amino acid residues in the antigenic peptide to enable binding into pockets defined by polymorphic residues along the class II binding site (7, 21). It is possible that the constant regions of the Opa protein have been selected for the presence of MHC-binding sequences which are presented to helper T cells efficiently. It seems likely that meningococci, for which humans are the only habitat, have adapted their OMPs to the human MHC in such a way that the least vulnerable proteins induce the highest immune response. Indeed, many positive responses were recorded for Opa peptides corresponding to conserved regions of the protein (Fig. 5). Finally, the immune response to the class 5 proteins might be modulated aspecifically, e.g., in an MHC-unrestricted fashion.

TABLE 2. Summary of human T-cell recognition of overlapping synthetic peptides spanning the sequence of class 5 and class 1 OMPs[a]

| Peptide | % of total number of peptides tested | | | |
|---|---|---|---|---|
| | Positive response | SI of 3–5 | SI of 5–10 | SI of >10 |
| OpaB | 36 | 19 | 10 | 8 |
| Opa5d | 24 | 12 | 8 | 4 |
| Opc | 21 | 14 | 6 | 1 |
| P1.7,16 | 23 | 15 | 6 | 3 |

[a] The individual responses observed for the class 5 protein-derived peptides are shown in Fig. 4. The peptides encompassing the P1.7,16 class 1 protein of reference strain H44/76 (32) were tested for recognition by PBMC in the same series of proliferation experiments (individual responses not shown). The total number of tests performed was 765 for OpaB, 666 for Opa5d, 720 for Opc, and 782 for P1.7,16.

Immunodominant T-cell epitopes derived from conserved regions of Opa proteins might be useful in the development of a meningococcal vaccine. However, in order to avoid genetically determined unresponsiveness to the vaccine, T-cell determinants that could be recognized in the context of the vast majority of MHC class II haplotypes should be incorporated. A highly polymorphic collection of HLA-DR and DQ gene products was expressed by the volunteers participating in this study. Several Opa peptides were found to be widely recognized, i.e., the OpaB peptides 6 to 8, 10 to 13, and 24 to 29 and the Opa5d peptides 19 to 23. The OpaB region from 124 to 147 and the homologous Opa5d region from 116 to 133 were capable of activating T cells obtained from all donors tested. Interestingly, these peptides correspond to sequences that are located outside the hypervariable regions (4, 9). Consequently, these T-cell epitopes may well be conserved among different meningococcal strains, indicating that they are likely to be common T helper antigenic sites.

The T-cell epitopes identified, in combination with B-cell-neutralizing determinants, may be useful in the development of a synthetic or recombinant meningococcal vaccine. Synthetic peptides encompassing such epitopes can be applied in B- and T-cell epitope-containing conjugate vaccines. The B-cell epitopes may be derived from the highly protective class 1 OMP, e.g., cyclic synthetic peptides mimicking protective epitopes occurring on surface loops (10). Furthermore, the T-cell epitopes can be conjugated with LPS-derived B-cell epitopes, e.g., inner core-derived oligosaccharides (6). Using this approach, a T-dependent B-cell response to these originally T-independent carbohydrate antigens may be elicited.

Alternatively, immunodominant T-cell determinants derived from class 5 proteins might be used to enhance the immunogenicity of outer membrane vesicle vaccines (molecular adjuvation). Membrane-spanning sequences of class 1 OMPs might be replaceable by more immunogenic class 5 sequences by genetic engineering, without disturbing the conformation of the class 1 protective surface loops.

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## REFERENCES

1. **Abdillahi, H., and J. T. Poolman.** 1988. *Neisseria meningitidis* group B serosubtyping using monoclonal antibodies in whole-cell ELISA. Microb. Pathogen. **4:**27–32.
2. **Achtman, M., M. Neibert, B. A. Crowe, W. Strittmatter, B. Kusecek, E. Weyse, M. J. Walsh, B. Slawig, G. Morelli, A. Moll, and M. Blake.** 1988. Purification and characterization of eight class 5 outer membrane protein variants from a clone of *Neisseria meningitidis* serogroup A. J. Exp. Med. **168:**507–525.
3. **Achtman, M., R. A. Wall, M. Bopp, B. Kusecek, G. Morelli, E. Saken, and M. Hassan-King.** 1991. Variation in class 5 protein expression by serogroup A meningococci during a meningitis epidemic. J. Infect. Dis. **164:**375–382.
4. **Aho, E. L., J. A. Dempsey, M. M. Hobbs, D. G. Klapper, and J. G. Cannon.** 1991. Characterization of the *opa* (class 5) gene family of *Neisseria meningitidis*. Mol. Microbiol. **5:**1429–1437.
5. **Barlow, A. K., J. E. Heckels, and I. N. Clarke.** 1989. The class 1 outer membrane protein of *Neisseria meningitidis*: gene sequence and structural and immunological similarities to gonococcal porins. Mol. Microbiol. **3:**131–139.
6. **Boons, G. J. P. H., P. Hoogerhout, J. T. Poolman, G. A. van der Marel, and**

**J. H. van Boom.** 1991. Preparation of a well-defined sugar-peptide conjugate: a possible approach to a synthetic vaccine against *Neisseria meningitidis*. Bioorg. Med. Chem. Lett. **1:**303–308.
7. **Brown, J. H., T. S. Jardetzky, J. C. Gorga, L. J. Stern, R. G. Urban, J. L. Strominger, and D. C. Wiley.** 1993. The three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature **364:**33–39.
8. **Feavers, I. M., J. Suker, A. J. McKenna, A. B. Heath, and M. C. J. Maiden.** 1992. Molecular analysis of the serotyping antigens of *Neisseria meningitidis*. Infect. Immun. **60:**3620–3629.
9. **Hobbs, M. M., A. Seiler, M. Achtman, and J. G. Cannon.** 1994. Microevolution within a clonal population of pathogenic bacteria: recombination, gene duplication and horizontal genetic exchange in the *opa* gene family of *Neisseria meningitidis*. Mol. Microbiol. **12:**171–180.
10. **Hoogerhout, P., E. M. L. M. Donders, J. A. M. van Gaans-van den Brink, A. J. Kuipers, H. F. Brugghe, L. M. A. van Unen, H. A. M. Timmermans, E. J. H. J. Wiertz, and J. T. Poolman.** Conjugates of synthetic cyclic peptides elicit bactericidal antibodies against a conformational epitope on a class 1 outer membrane protein of *Neisseria meningitidis*. Manuscript submitted for publication/Infect. Immun. ASM control number IAI 14-95.
11. **Maiden, M. C. J., J. Suker, A. J. McKenna, J. A. Bygraves, and I. M. Feavers.** 1991. Comparison of the class 1 outer membrane proteins of eight serological reference strains of *Neisseria meningitidis*. Mol. Microbiol. **5:**727–736.
12. **Mandrell, R. E., and W. D. Zollinger.** 1989. Human immune response to meningococcal outer membrane protein epitopes after natural infection or vaccination. Infect. Immun. **57:**1590–1598.
13. **McGuinness, B., A. K. Barlow, I. N. Clarcke, J. E. Farley, A. Anilionis, J. T. Poolman, and J. E. Heckels.** 1990. Comparative sequence analysis of the class 1 protein gene (por A) from three strains of *Neisseria meningitidis*: sythetic peptides define the epitopes responsible for serosubtype specificity. J. Exp. Med. **171:**1871–1882.
14. **Olyhoek, A. J. M., J. Sarkari, M. Bopp, G. Morelli, and M. Achtman.** 1991. Cloning and expression in *Escherichia coli* of *opc*, the gene for an unusual class 5 outer membrane protein from *Neisseria meningitidis*. Microb. Pathogen. **11:**249–257.
15. **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.
16. **Poolman, J. T., C. T. P. Hopman, and H. C. Zanen.** 1983. Immunogenicity of meningococcal antigens as detected in patient sera. Infect. Immun. **40:**398–406.
17. **Poolman, J. T., J. A. M. Timmermans, T. Teerlink, and R. C. Seid, Jr.** 1989. Purification, cyanogen bromide cleavage, and amino terminus sequencing of class 1 and class 3 outer membrane proteins of meningococci. Infect. Immun. **57:**1005–1007.
18. **Rosenqvist, E., E. A. Høiby, E. Wedege, B. Kusecek, and M. Achtman.** 1993. The 5C protein of *Neisseria meningitidis* is highly immunogenic in humans and induces bactericidal antibodies. J. Infect. Dis. **167:**1065–1073.
19. **Sarkari, J., N. Pandit, E. R. Moxon, and M. Achtman.** 1994. Variable expression of the Opc outer membrane protein in *Neisseria meningitidis* is caused by size variation of a promoter containing poly-cytidine. Mol. Microbiol. **13:**207–217.
20. **Saukkonen, K., M. Leinonen, H. Abdillahi, and J. T. Poolman.** 1989. Comparative evaluation of potential components for group B meningococcal vaccine by passive protection in the infant rat and *in vitro* bactericidal assay. Vaccine **7:**325–328.
21. **Stern, L. J., J. H. Brown, T. S. Jardetzky, J. C. Gorga, R. G. Urban, J. L. Strominger, and D. C. Wiley.** 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature **368:**215–221.
22. **Suker, J., I. M. Feavers, M. Achtman, G. Morelli, J.-F. Wang, and M. C. J. Maiden.** 1994. The *porA* gene in serogroup A meningococci: evolutionary stability and mechanism of genetic variation. Mol. Microbiol. **12:**253–265.
23. **Tinsley, C. R., and J. E. Heckels.** 1986. Variation in the expression of pili and outer membrane protein by *Neisseria meningitidis* during the course of meningococcal infection. J. Gen. Microbiol. **132:**2483–2490.
24. **Tommassen, J., P. Vermeij, M. Struyvé, R. Benz, and J. T. Poolman.** 1990. Isolation of *Neisseria meningitidis* mutants deficient in class 1 (PorA) and class 3 (PorB) outer membrane proteins. Infect. Immun. **58:**1355–1359.
25. **Tsai, C.-M., C. E. Frasch, and L. F. Mocca.** 1981. Five structural classes of major outer membrane proteins in *Neisseria meningitidis*. J. Bacteriol. **146:**69–78.
26. **Van der Ley, P., J. E. Heckels, M. Virji, P. Hoogerhout, and J. T. Poolman.** 1991. Topology of outer membrane porins in pathogenic neisseria. Infect. Immun. **59:**2963–2971.
27. **Virji, M., K. Makepeace, D. J. P. Ferguson, M. Achtman, and E. R. Moxon.** 1993. Meningococcal Opa and Opc proteins: their role in colonization and invasion of human epithelial and endothelial cells. Mol. Microbiol. **10:**499–510.
28. **Virji, M., K. Makepeace, D. J. P. Ferguson, M. Achtman, J. Sarkari, and E. R. Moxon.** 1992. Expression of the Opc protein correlates with invasion of epithelial and endothelial cells by *Neisseria meningitidis*. Mol. Microbiol. **6:**2785–2795.
29. **Wang, J.-F., D. A. Caugant, X. Li, X. Hu, J. T. Poolman, B. A. Crowe, and M. Achtman.** 1992. Clonal and antigenic analysis of serogroup A *Neisseria meningitidis*, with particular reference to epidemiological features of epidemic meningitis in the People's Republic of China. Infect. Immun. **60:**5267–5282.
30. **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.
31. **Wedege, E., and T. E. Michaelsen.** 1987. Human immunoglobulin G subclass immune response to outer membrane antigens in meningococcal group B vaccine. J. Clin. Microbiol. **25:**1349–1353.
32. **Wiertz, E. J. H. J., J. A. M. van Gaans-van den Brink, H. Gausepohl, A. Prochnicka-Chalufour, P. Hoogerhout, and J. T. Poolman.** 1992. Identification of T cell epitopes occurring in a meningococcal class 1 outer membrane protein using overlapping peptides assembled with simultaneous multiple peptide synthesis. J. Exp. Med. **176:**79–88.
33. **Wiertz, E. J. H. J., J. A. M. van Gaans-van den Brink, G. M. T. Schreuder, A. A. M. Termijtelen, P. Hoogerhout, and J. T. Poolman.** 1991. T cell recognition of *Neisseria meningitidis* class 1 outer membrane proteins: identification of T cell epitopes with selected synthetic peptides and determination of HLA restriction elements. J. Immunol. **147:**2012–2018.
34. **Woods, J. P., and J. G. Cannon.** 1990. Variation in expression of class 1 and class 5 outer membrane proteins during nasopharyngeal carriage of *Neisseria meningitidis*. Infect. Immun. **58:**569–572.

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