Potential of Recombinant Opa Proteins as Vaccine Candidates against Hyperinvasive Meningococci

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Neisseria meningitidis causes half a million cases of septicaemia and meningitis globally each year. The opacity (Opa) integral outer membrane proteins from N. meningitidis are polymorphic and highly immunogenic. Particular combinations of Opa proteins are associated with the hyperinvasive meningococcal lineages that have caused the majority of serogroup B and C meningococcal disease in industrialized countries over the last 60 years. For the first time, this genetic structuring of a diverse outer membrane protein family has been used to select a novel combination of representative antigens for immunogenicity testing. Fourteen recombinant Opa variants were produced and used in murine immunizations inducing an increase in specific antimenningococcal total IgG levels. All 14 Opa proteins elicited bactericidal antibodies against at least one hyperinvasive meningococcal isolate, and most isolates from each hyperinvasive lineage were killed by at least one Opa antiserum at a titer of 1:16 or greater. Cross-reactive bactericidal antibody responses were observed among clonal complexes. A theoretical coverage of 90% can be achieved by using a particular combination of 6 Opa proteins against an isolate collection of 227 recent United Kingdom disease cases. This study indicates the potential of Opa proteins to provide broad coverage against multiple meningococcal hyperinvasive lineages.

Neisseria meningitidis is a pathogen of global importance, causing 500,000 cases of meningococcal disease worldwide each year, with up to 6 cases per 100,000 in Europe, and a mortality rate of approximately 10% (42, 52). Safe and effective vaccines based on the meningococcal serogrouping antigen, the capsular polysaccharide, are available against four of the five serogroups (A, C, W135, and Y) that commonly cause disease (34). The poor immunogenicity of the serogroup B capsular polysaccharide and its antigenic similarity to saccharides on the surface of human cells have, however, hindered the development of a serogroup B polysaccharide vaccine (16, 17, 53). This has prompted the evaluation of a number of noncapsular antigens, but none of these have yet provided broad protection against meningococci commonly associated with disease, due to the antigenic heterogeneity of this species. Population studies suggest that combinations of opacity-associated adhesin (Opa) proteins, whose vaccine candidacy had previously been rejected on the basis of their antigenic diversity, may provide coverage against a range of meningococcal strains (5).

Opa proteins are one of the major groups of proteins found in the meningococcal outer membrane. The four opa loci opaA, opaB, opaD, and opaJ are constitutively transcribed, with expression controlled at the translational level by changes in the length of a pentameric repeat tract within the open reading frame of the gene, located in the leader peptide sequence between the start codon and the first codon of the mature polypeptide (40). Opa proteins play an important role in initial colonization by mediating intimate adhesion to epithelial cells via interactions with heparin sulfate proteoglycans and members of the carcioembryonic antigen cell adhesion molecule (CEACAM) family (32, 33, 48). Opa proteins exhibit a high level of antigenic diversity due to sequence variation in three of the four surface-exposed loops, including a semivariable (SV) region in loop 1 and two hypervariable regions (HV1 and HV2) in loops 2 and 3, respectively (12, 28, 45). These regions, in particular, HV1 and HV2, also mediate receptor tropism (33, 46). Anti-Opa IgG antibodies, including bactericidal antibodies, have been demonstrated in patients following meningococcal infection and in recipients of serogroup B outer membrane vesicle (OMV) vaccines, suggesting that Opa proteins are immunogenic in humans (29, 31, 38).

Despite the genetic and antigenic diversity of meningococci isolated from asymptomatic carriers, the majority of invasive meningococcal disease over the past 6 decades has been attributed to fewer than 10 groups of related meningococci (clonal complexes), known as the “hyperinvasive lineages” (9, 25). Before the recent emergence of the sequence type 269 (ST-269) complex (14, 23), as few as four clonal complexes

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In this study, the potential of combinations of Opa proteins as meningococcal vaccine candidates was evaluated by immunizations in mice with recombinant Opa proteins from the hyperinvasive lineages. Bactericidal antibodies were elicited against isolates belonging to the ST-8, ST-11, ST-32, and ST41/44 clonal complexes. In addition, cross-reactive anti-Opa antibody responses were observed between clonal complexes.

**MATERIALS AND METHODS**

Choice of meningococcal isolates. Isolates were collected from diverse geographic and temporal origins and belonged to four hyperinvasive lineages: the ST-8, ST-11, ST-32, and ST41/44 clonal complexes (25) (Table 1). The Opa repertoire of these meningococci has been described (5), and 14 Opa genes were chosen to produce recombinant Opa proteins (Table 2).

Bacterial growth conditions. Meningococci were grown on brain heart infusion agar, supplemented with defibrinated horse blood, at 37°C in a humidified 5% CO<sub>2</sub> atmosphere. The Escherichia coli strains TOP10 and BL21*DE3 (Invitrogen, Carlsbad, CA) were used to propagate recombinant DNA constructs and for recombinant protein expression, respectively. E. coli was grown in Luria-Bertani (TOP10) or double yeast tryptone (BL21*DE3) medium supplemented with ampicillin (50 μg/ml).

Construction of pET22b(+)/plasmids. Opa loci were amplified from genomic meningococcal DNA first using a locus-specific PCR as previously described (5) and then by a generic PCR using primers which introduced novel NdeI and XhoI restriction sites (underlined): opaNDdeI (5′-GAAGTCTCTCGCGACGGCAATCCGCGAATAAGAGA-3′) and opaXhoIR (5′-GGAATTCTCTCGACGACGGCAATCCGCGAATAAGAGA-3′). The opaNDdeI primer also introduced a new initiation codon immediately upstream of the first codon of the mature polypeptide sequence, thereby freeing the new ampiclon from the coding repeat tract to avoid the problem of phase variation. Amplicons were ligated into Escherichia coli strains TOP10 and BL21*DE3, chosen to produce recombinant Opa proteins (Table 2).
the plasmid pCR2.1 (Invitrogen, Carlsbad, CA) and used to transform E. coli TOP10. Recombinant plasmids were purified using a QiAprep kit (Qiagen, Crawley, United Kingdom), and the opa gene was excised using NdeI and XhoI. The expression plasmid pET22b (+) (Novagen, Whitehouse Station, NJ) was similarly prepared prior to ligation, together with Φ2 DNA ligase (Calbiochem, Whitehouse Station, NJ). Ligation reactions were used to transform E. coli TOP10, and the plasmids were purified as before. The opa sequence in the pET22b (+)/opa plasmids constructs was confirmed to be identical to the appropriate original sequence. The pentameric repeat tract of each opa gene was determined by nucleotide sequencing as described previously (5) but using primers MH4 (previously described) and OpaFwdII (5′-TATATTGTGTGGTAACACATCG-3′).

**TABLE 2. Opa protein antigens used in this investigation**

<table>
<thead>
<tr>
<th>Clonal complex</th>
<th>Cloning isolate</th>
<th>Protein</th>
<th>opa allele</th>
<th>HV1</th>
<th>HV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-8 complex</td>
<td>94/155</td>
<td>Opa Ab</td>
<td>opa246 (3/6)</td>
<td>15-3</td>
<td>12-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaD</td>
<td>opa161 (2/6)</td>
<td>11-1</td>
<td>1-4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaJ</td>
<td>opa246 (2/6)</td>
<td>15-3</td>
<td>12-2</td>
</tr>
<tr>
<td>ST-11 complex</td>
<td>38V1</td>
<td>OpaA</td>
<td>opa244 (6/8)</td>
<td>5-5</td>
<td>8C-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaB</td>
<td>opa34 (2/8)</td>
<td>18-3</td>
<td>14-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaC</td>
<td>opa132 (8/8)</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaJ</td>
<td>opa317 (5/8)</td>
<td>19-1</td>
<td>14-1</td>
</tr>
<tr>
<td>ST-32 complex</td>
<td>BZ169</td>
<td>OpaA</td>
<td>opa96 (8/8)</td>
<td>19-10</td>
<td>14-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaB</td>
<td>opa185 (4/8)</td>
<td>10-5</td>
<td>3-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaD</td>
<td>opa147 (6/8)</td>
<td>1A-2</td>
<td>8-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaJ</td>
<td>opa218 (8/8)</td>
<td>19-10</td>
<td>14-3</td>
</tr>
<tr>
<td>ST-41/44</td>
<td>BZ198 88/03415</td>
<td>OpaA</td>
<td>opa245 (2/7)</td>
<td>1-5</td>
<td>9A-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaB</td>
<td>opa201 (3/7)</td>
<td>4-1</td>
<td>7-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaD</td>
<td>opa265 (2/7)</td>
<td>7-1</td>
<td>5-9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OpaJ</td>
<td>opa213 (4/7)</td>
<td>3-6</td>
<td>1-1</td>
</tr>
</tbody>
</table>

a The antigens were chosen on the basis of the frequency of their immunodominant hypervariable region combinations in the ST-8, ST-11, ST-32, and ST-41/44 complexes. The most common variants in the semivariable region were also covered by these antigens. The numbers in the HV1 and HV2 columns denote the variant of the hypervariable region, according to the nomenclature of Collaghan et al. (5). The ST-41/44 complex opaB was amplified for cloning from 88/03415, whereas all other loci from this complex were amplified from another member of the ST-41/44 complex, BZ198.
b The same allele is present at both the opaA and opaD loci in this isolate, and so only one protein was produced to cover these.
c Numbers in parentheses represent the number of isolates in that clonal complex that possess the allele at that locus/total number in that clonal complex.

d 2% skim milk powder in phosphate-buffered saline. Pooled sera from an immunized mouse (diluted 1 in 20, 1 in 200, and 1 in 2,000) were added prior to incubation with anti-mouse IgG-Fc antibody conjugated to alkaline phosphatase (Sigma). Plates were developed in the dark following the addition of alkaline phosphatase yellow (p-nitrophenyl phosphate [pNPP]) liquid substrate system (Sigma) for 5 min, before the reaction was stopped using 3 M NaOH and the optical density at 620 nm was recorded. Each serum pool was analyzed in duplicate at all three dilutions.

Immunogold transmission electron microscopy (TEM) of binding of anti-Opa antibody to meningococci. A negative-staining technique using methyl tungstate was used as previously described (47). Opa expression on the bacterial surface was visualized by binding of polyclonal anti-Opa murine sera and detection by goat anti-mouse Ig conjugated to colloidal gold particles.

SBA assay. Bactericidal activity in pooled mouse serum from the same group was quantified by serum bactericidal antibody (SBA) assay. Briefly, pooled murine serum was heated at 56°C for 30 min to deactivate endogenous complement and then diluted to give a range from 1:4 to 1:4,096. Diluted serum was incubated with meningococci (1,000 CFU) for 10 min, before the addition of baby rabbit complement (lot number 20128; PelFreez Biologicals, Rogers, AR) at a final concentration of 1:16. Reaction mixtures were incubated for 1 h at 37°C in a humidified 5% CO2 atmosphere. A sample of each reaction mixture was spread on a plate of cefsulodin-irgucitin-biotin (CIB) agar medium containing 5% horse serum and 4% defibrinated sheep blood, and the colonies were counted. Each serum sample was analyzed in duplicate at all three dilutions.

**Cross-reactivity of anti-Opa antibodies.** Serum cross-reactivity was analyzed using immunodot blotting as an initial screen. Ethanol-fixed meningococcal cell suspensions were dried overnight onto a nitrocellulose membrane. Pooled murine serum was then added at a dilution of 1:200, and antibody binding was detected using anti-mouse IgG monoclonal antibody conjugated to alkaline phosphatase and 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium solution (Perkin Elmer, Waltham, MA). The color was allowed to develop until the background signal developed, and the intensity of the color was visually rated by two independent operators.

Coverage of clonal complexes and Opa diversity in serogroup B-associated hyperinvasive_lineages. The HV1 and HV2 regions of the 14 Opa proteins in this study were compared with those of a collection of 227 isolates from invasive meningococcal disease in the United Kingdom collected between 1996 and 2001 (6) to evaluate the potential coverage of an Opa protein vaccine. The analysis was repeated by considering the variable bactericidal antibody responses elicited by each protein.
RESULTS

Purification of refolded, recombinant Opa proteins. A total of 14 Opa proteins were chosen on the basis of the opa repertoire of a collection of 29 diverse meningococcal isolates from four hyperinvasive lineages and expressed in E. coli. The most commonly occurring opa genes from each clonal complex were included, and these were present in 17 to 100% of homologous opa loci within the same clonal complex (Table 2). Cell lysates of isopropyl-β-D-thiogalactopyranoside-induced E. coli bearing a pET22b(+)/opaplasmid contained an additional polypeptide of approximately 28 kDa, which was subsequently identified to be Opa by mass spectrometry (see Fig. S1A in the supplemental material). The refolded, affinity-purified protein preparations contained >95% Opa protein together with some minor contaminants from the E. coli expression host. Immunoblotting demonstrated that murine antisera raised against this protein preparation were specific for Opa in the meningococcal cell lysate (see Fig. S1B in the supplemental material).

Antibody responses to meningococcal cell lysates. The immunogenicity of these 14 Opa proteins was evaluated by analysis of murine postvaccination sera using ELISA against meningococcal cell lysates derived from 6 to 8 isolates within the same clonal complex. Fold rise in total IgG levels was determined by comparison of sera from mice immunized with Opa protein to sera from those immunized with Freund’s adjuvant alone. For each Opa protein, the dose which gave the highest fold rise in total IgG was used for further analysis. No consistent effect of the dose on the fold rise in IgG was observed. For each of the 29 isolates, all sera obtained after immunization with an Opa protein from the same clonal complex were tested in the ELISA (Fig. 1). The highest fold rise for each isolate was greater than 2 for 100% of isolates and greater than 3, 4, and 5 for 79%, 72%, and 62% of isolates, respectively. The median fold rise in ELISA titer was 5.60. Replicate experiments demonstrated good reproducibility of assays between plates, over time, and between operators (data not shown).

Bactericidal antibody against meningococcal Opa proteins. Murine postvaccination sera were used in an SBA assay with 6 to 8 isolates from the same clonal complex as the Opa protein used in the immunization (Table 1). The majority of these isolates possessed the same alleles as the immunizing strain (Table 2). Expression of Opa proteins, an essential prerequisite for anti-Opa bactericidal activity, was assessed by analysis of the nucleotide sequence of the coding repeat tract of all opa genes to determine whether the gene was likely to be expressed. Of the 29 isolates, 27 had at least one Opa protein both expressed and targeted. Of these 27, 24 were killed in the SBA by at least one serum sample, and all isolates belonging to the ST-11 and ST-32 complexes with an Opa expressed and targeted were killed (Fig. 2A). The highest SBA titer for each isolate was greater than 16 in 100% of isolates and greater than...
64, 256, and 512 in 96%, 88%, and 83% of isolates, respectively (Fig. 2B). Overall, the median SBA titer was 1,659. Among the isolates tested that expressed the Opa proteins corresponding to those used in the immunization, 100% of the ST-11 and ST-32 clonal complexes, 83% of the ST-8 complex, and 71% of the ST-41/44 complex were killed by antibodies raised against at least one Opa protein from the same clonal complex. Bactericidal responses were sometimes not observed even when antibody was detected by ELISA. Within groups of mice, SBA titers were similar and within 2 dilutions (data not shown). No consistent effect of the dose on the SBA titer was observed. Replicate experiments demonstrated good reproducibility of assays between plates, over time, and between operators (data not shown).

Electron microscopic detection of binding of anti-Opa antibody to meningococci. Combinations of sera and isolates which gave a range of results in the SBA assay (i.e., high, medium, and low titers) were chosen for detection of antibody binding by TEM. Binding of pooled murine sera raised against the OpaB185 protein from the ST-32 complex against the meningococcal isolate BZ83 from the same clonal complex was visualized by TEM (Fig. 3). This was compared with binding against isolate H44/76, the Opa proteins of which were not expected to be expressed. No antibody binding was observed against H44/76. Pooled sera raised against the OpaB protein from most ST-11 complex isolates gave a high SBA titer with isolate 0037/93 and no killing against NGP20, which were also corroborated by TEM, which revealed high reactivity of this sera against 0037/93 and very low reactivity against NGP20 (data not shown).

Cross-reactive bactericidal antibodies against other clonal complexes. Although isolates expressing multiple Opa proteins did not appear to elicit higher titers of bactericidal antibodies, suggesting that there was limited cross-reactivity, this possibility was explored further. Ethanol-fixed meningococcal suspensions of all isolates were dot blotted against all postvaccination sera from 50-μg dose immunizations to identify cross-reactivity of the anti-Opa response. All sera were tested against all isolates used in this study (Table 1). Of the 406 combinations tested, 25 gave a positive result in the dot blot and 14 were randomly chosen to assess SBA activity. Five of these serum-isolate combinations also generated a bactericidal response in the SBA assay (Fig. 4). In addition, 8 combinations which gave little or no response in the dot blot were assessed for SBA activity to examine cross-reactivity between a range of clonal complexes. Only one of these eight resulted in bacteriolysis of the isolate tested. Where a cross-reactive bactericidal response was detected, all the titers elicited were greater than 1:128 (Fig. 4).

Coverage of clonal complexes and Opa diversity in serogroup B-associated hyperinvasive lineages. For overall coverage, it was assumed that for any isolate with an HV1 or HV2 region (in any of its Opa proteins) identical to that of the immunizing Opa, the result could be bacteriolysis (described from here on as a “hit”). The Opa protein with the highest coverage, i.e., the highest number of matching HV1 or HV2 regions in this collection, was considered to be the initial potential vaccine component. Subsequent Opa proteins were added sequentially, ordered to maximize the total coverage. Comparison of the HV1 and HV2 regions of the 14 Opa proteins studied here with a collection of 227 isolates from invasive meningococcal disease in the United Kingdom between 1996 and 2001 (6) indicated the potential coverage of an Opa protein vaccine both theoretically and after adjustment to include the SBA results described above, i.e., reducing predicted coverage on the basis of the finding that not all targeted isolates were killed (Fig. 5). The OpaD protein from the ST-11 clonal complex (Opa132) contained the most commonly occurring HV1 and HV2 regions in this collection, with a coverage of 49% of the isolates. Increasing the number of proteins in a putative Opa vaccine to six increased theoretical coverage to 90%. Coverage with two or more hits per organism, on either HV1 or HV2 from any one of the four Opa proteins, increased from 29% with 1 Opa protein up to 78% with 6 components (Fig. 5). Taking into account that antisera were not bactericidal
for all targeted isolates, the estimated maximum coverage would be 69% with 6 Opa proteins for this isolate collection.

The potential coverage of individual clonal complexes by a single Opa protein from the same complex, with two hits per Opa protein, on both the HV1 and HV2 region ranged from 65% in the ST-32 complex to 100% in the ST-8 complex. With a single hit per Opa protein on either the HV1 or HV2 region, the range increased from 85% in the ST-41/44 complex to 100% in the ST-8 complex.

**DISCUSSION**

No vaccine has yet provided universal protection against serogroup B meningococcal disease. In the absence of a safe and immunogenic polysaccharide vaccine, outer membrane vesicle vaccines have been widely used but are often efficacious against only the target strain. To increase coverage, outer membrane protein (OMP) vaccines comprising many variants of a single protein have been considered. However, the high

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**FIG. 4.** Summary of dot blotting and SBA analyses to assess cross-reactivity of bactericidal antibodies against isolates from clonal complexes other than that of the Opa protein used for immunization. The central box provides details of the Opa protein used for immunization and the target isolate against which bactericidal antibodies were demonstrated, with the associated SBA titer. *, positive dot blots were those rated “high-intensity” reactions by two independent observers; **, negative when tested by dot blotting; CC, clonal complex of Opa protein or target strain.

**FIG. 5.** Increasing potential vaccine coverage (hypothetical and adjusted for SBA results) of 227 isolates from invasive disease in the United Kingdom between 1996 and 2001 (at least one Opa protein in their repertoire was targeted) with addition of each Opa variant by ranked frequency, showing the number of hits (i.e., HV1 or HV2) per organism. One hit occurs when an HV1 or HV2 region of an isolate that is identical to the HV1 or HV2 region from one of the Opa proteins. In this example, Opa proteins would be added in this order: 1, Opa132; 2, Opa213; 3, Opa96; 4, Opa246; 5, Opa317; 6, Opa201; 7, Opa265; 8, Opa245; 9, Opa161; 10, Opa34; 11, Opa185; 12, Opa147; 13, Opa244; 14, Opa218.
diversity of subcapsular antigens has been an impediment to the development of this approach. Therefore, the challenge of developing OMP vaccines, including an Opa-based vaccine, is 2-fold: the first is to have protein variants that are immunogenic, and the second is to include all variants needed for broad coverage but still keep the number of variants small enough so as to make a practicable vaccine. In this study, proteins from a representative panel of 14 recombinant Opa proteins from four hyperinvasive lineages have been shown to be immunogenic in mice and elicit bactericidal antibodies against a temporally and geographically diverse strain collection, supporting further development of a recombinant Opa vaccine.

In assessing the likely potential of meningococcal vaccines, demonstration of SBA responses is currently the "gold standard" (3, 50). After immunization of mice with each of the 14 Opa proteins, 89% of isolates which expressed the correct Opa variant were killed by at least one anti-Opa serum in the SBA assay. In this study, after immunization with each Opa protein, murine bactericidal antibodies were elicited against at least one isolate expressing the antigenically identical protein (i.e., identical amino acid sequence), confirming their immunogenicity.

Despite variable SBA responses in some isolates, bactericidal antibodies were elicited after immunization with all proteins used in this investigation and were observed against isolates belonging to the same clonal complex but expressing different capsular polysaccharides. Consequently, immunization with hyperinvasive lineage-specific combinations of Opa proteins may circumvent the effects of meningococcal capsule switching which can occur as a result of horizontal genetic exchange of the siaD gene (41). With the widespread use of vaccines based on the A, C, Y, and W-135 capsular polysaccharides, capsule replacement could be an important mechanism of meningococcal escape from vaccine-induced antibody responses in the future. For example, meningococci from the hyperinvasive ST-11 complex expressing the serogroup B polysaccharide have been isolated from patients following a serogroup C conjugated-polysaccharide vaccination campaign (7). Furthermore, the invasiveness of this clonal complex has been shown to remain unchanged after it switched from expression of a serogroup C capsule to serogroup B (22). It is possible that a vaccine containing Opa proteins could promote the emergence of vaccine escape variants since a wide diversity of Opa variants occurs in both pathogenic and commensal Neisseria strains. However, the structured diversity of Opa alleles that occurs in hyperinvasive lineages suggests that these particular Opa variants are associated with hyperinvasive lineages, and if such evolution occurs, the resulting clones may be less likely to cause invasive disease.

Another potential advantage of using Opa proteins as vaccines is that their use may lead to the generation of herd immunity. Opa proteins are important in mediating intimate contact with the host, and immunization with Opa proteins may elicit the production of antibodies that could block adhesion and so carriage and subsequently transmission. Antibodies against Opa proteins from Neisseria gonorrhoeae correlated with a reduced risk of salpingitis perhaps by interfering with Opa-mediated adhesion (35). Furthermore, herd immunity is known to be a major factor in the control of serogroup C meningococcal disease in the United Kingdom, and it is likely that the effectiveness of a novel meningococcal vaccine would be substantially enhanced if it were able to reduce colonization (26, 36). Anti-Opa IgG antibody was demonstrated in postvaccination sera, even in the absence of bactericidal activity, and such antibodies may still be helpful in contributing toward a herd immune effect with an Opa vaccine. Any herd immune effect also offers the potential to protect younger infants who have not received a complete immunization course. As the number of proteins in an Opa vaccine is increased, there is a concomitant rise in the number of epitopes targeted on the surface of any given organism, highlighted by the coverage data described above. It is therefore possible that antibodies which are not bactericidal independently may result in bacteriolysis by acting synergistically with other anti-Opa antibodies, as has been suggested for factor H binding protein (fHbp) (1, 49).

Several different approaches to a protein-based meningococcal vaccine have been investigated previously (11, 20), but, excluding OMV-based vaccines, just two have progressed beyond phase I clinical trials. An fHbp vaccine containing 2 variants of fHbp and a vaccine containing a combination of 4 components identified from the meningococcal genome (4CMenB) are currently in development (18), the latter being in phase III clinical trials (15, 39). An alternative approach is production of a vaccine from combinations of different variants of variable antigens, such as Opa. The advantage of such antigens is that they are under strong immune selection, the reason for their variability, and so are more likely to elicit a good antibody response. This approach is similar to that used in the production of hexavalent and nonvalent PorA OMV vaccines (10, 44). Although constant diversification of immunogenic outer membrane antigens may lead to vaccine escape, such that further development of these vaccines to reflect the changing epidemiology may well be necessary, the approach adopted in the present study has been shown to elicit bactericidal responses against the vaccine components (8, 13).

Expression of Opa proteins is phase variable and is controlled by variation in the length of a pentameric coding repeat within the open reading frame of Opa genes (21). Variation in the promoters of Opa genes in the related bacterium Neisseria gonorrhoeae also affects both the expression level of Opa proteins and the rate of phase variation (2). Our nucleotide sequence data confirm that Opa phase variation among the meningococci targeted in the SBA assay is controlled at the translational level, although further mechanisms may also result in variation in expression levels and also therefore affect vaccine coverage. In addition, not all isolates possessed all Opa genes encoding proteins used in immunizations and so even if they were expressed were not likely to be targeted by Opa-specific antibodies. The effect of various expression levels in target isolates may affect the assessment and effectiveness of other meningococcal candidate antigens, many of which are also encoded by phase-variable genes. However, by including as many Opa proteins from a clonal complex as possible in a prospective vaccine, the effect of phase variation, reduced expression levels, and potential escape from vaccine-induced immune responses might be counteracted.

T cell responses are likely to play an important role in the generation of an optimal immune response, and the meningococcus appears to modulate host T cell responses through the
Opa proteins. Some Opa proteins also allow meningococcal interaction with neutrophils (48) and other phagocytes (30), whereas gonococcal Opa proteins stimulate macrophages to produce proinflammatory cytokines (27). Purified Opa proteins have elicited strongly proliferative T cell responses (51). Conversely, others have found that Opa proteins expressed on the surface of gonococci (4) arrest activation and proliferation of CD4+ T cells via a CEACAM1-mediated mechanism (24). Data from studies using meningococcal OMVs have yielded conflicting results (24, 54). More data on the interaction between Opa proteins and CD4+ T cells are therefore required.

There are some limitations to the use of these data in assessing the potential for the use of Opa proteins as a vaccine in humans. First, Freund’s is a potent adjuvant which is not licensed for use in humans and tends to produce high titers. Now that we have demonstrated proof in principle of using Opa proteins as a potential meningococcal vaccine, it is necessary to consider other adjuvants in future studies. Second, recent data suggest that rabbit complement factor H (fH) does not bind to N. meningitidis, unlike human fH. Binding of fH to the bacterial surface downregulates complement activation, leading to higher SBA titers and overestimation of vaccine efficacy with rabbit complement (19). The optimum dose for immunization is yet not clear, as some proteins induced a better response with 5 μg and some did so with 50 μg. Finally, mouse studies would not necessarily provide clarity regarding T cell immune responses due to the differences in CEACAMs found in mice compared to humans (http://www.carcinoembryonic-antigen.de, accessed 20 February 2011).

In this study we have shown for the first time that immunization with recombinant Opa proteins elicits the production of serum bactericidal antibodies and have demonstrated that 14 of the most prevalent Opa proteins are highly immunogenic in a mouse model, offering the potential for a meningococcal vaccine targeting the hyperinvasive lineages. However, further information is required to more fully understand the adhesive and immunomodulatory activities of the Opa proteins and their roles in meningococcal colonization and pathogenesis, prior to human trials. It is possible that Opa proteins could ultimately be included in a vaccine alongside current capsular polysaccharide antigens, to provide broad protection against meningococcal disease.

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