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Effect of sequence variation in meningococcal PorA outer membrane protein on the effectiveness of a hexavalent PorA outer membrane vesicle vaccine in toddlers and school children

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Abstract

Though meningococcal conjugate vaccines are effective against serogroup C, there is currently no vaccine solution for serogroup B disease. PorA outer membrane protein (OMP) is a potential serogroup B vaccine candidate. A hexavalent PorA outer membrane vesicle (OMV) vaccine has been evaluated in phase I and II trials with promising results. However, considerable sequence variation occurs in the variable regions (VRs) encoding these serosubtypes. By using five wild type P1.19,15 variant strains we examined the serum bactericidal antibody (SBA) titres from sera collected from toddlers and school children pre- and post-vaccination. The numbers of subjects with SBA titres of <4, 4 and ≥8 varied greatly between the different strains. This was also reflected when ≥4-fold rises in SBA titres were examined. This finding in sera from toddlers and school children may have implications for PorA based vaccines.

Keywords: Meningococcal PorA; Hexavalent PorA; Outer membrane vesicle

1. Introduction

Meningococcal disease remains a major international health problem. Meningococcal polysaccharide-protein conjugate vaccines for serogroup C have demonstrated their effectiveness in significantly reducing serogroup C disease in the UK [1,2]. Meningococcal conjugate vaccines are now advanced for serogroup A [3] and for serogroups A,C,Y,W135 as a tetravalent conjugate vaccine [4]. However, due to the poor immunogenicity of the serogroup B capsular polysaccharide in humans [5] and fears of inducing autoimmune antibodies due to antigenic similarities with human neural cell adhesion molecules [6], the approach of modifying the serogroup B polysaccharide is proving difficult [7,8].

Several candidate outer membrane vesicle (OMV) vaccines have been developed and subjected to large-scale efficacy studies in Norway, Cuba, Brazil and Chile [9–12]. Each vaccine was formulated from a locally prevalent epidemic strain and contained the major porins with additional high molecular weight outer membrane proteins (OMPs). Efficacy estimates varied from 57 to 83% in those aged over 4 years, but no protection was demonstrated in children aged less than 2 years. A scattered range of immune responses was observed amongst recipients, complicating the analysis of the specificity of the induced bactericidal antibodies. However,

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links between PorA OMP-specific antibodies and bactericidal activity have been noted [13,14].

Currently there is a lack of information about serological correlation with protection for serogroup B, however the serum bactericidal antibody (SBA) assay has recently been evaluated as the appropriate correlate of protection. Sera from the Norwegian serogroup B efficacy trial has been reanalysed resulting in the proposal of a tentative protective SBA titre of ≥4 [15]. Previously most studies of serogroup B OMV vaccines have used ≥4-fold rises from pre- to post-vaccination and not relied on absolute SBA titres as a cut off. This approach may be more rational due to the inherent variation in the SBA assay between laboratories, yet the percentage with ≥4-fold rises remains relatively constant [16,17].

A hexavalent PorA OMV vaccine has been developed at the National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands in which six PorA OMPs are embedded in OMVs (P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13 and P1.7-2,4) [18]. Immunogenicity studies have demonstrated that the vaccine is safe, well tolerated and immunogenic in English infants [19], and in Dutch toddlers and school children [20], although multiple doses of vaccine are required to induce significant serum bactericidal activity. However, a much larger number of variants of individual serosubtypes have been identified [21] and concerns have been raised that antibodies raised against one particular variant may fail to promote complement-mediated bactericidal killing of another variant [22,23].

In the UK in 2000/2001 the most prevalent disease causing serosubtype was 7-2, 4, accountable for 31.8% of serogroup B disease [24] and which is contained within the hexavalent vaccine formulation. Serosubtypes 19-1,15-11; 19,15 and 19,15-1 accounted for 13.0, 5.7 and 1.25%, respectively, of serogroup B disease [24], of which only 19,15-1 is contained within the hexavalent vaccine formulation. Therefore, as 18.7% of UK serogroup B, P1.15 disease is due to variants not contained within the current hexavalent vaccine formulation we investigated the cross-reactivity of antibody induced by the PorA component P1.19,15-1 against wild type P1.19,15 variants in pre- and post-vaccination sera collected from Dutch toddlers and school children [20].

2. Methods

2.1. Sera

Sera containing bactericidal antibodies (≥4) against an isogenic P1.19,15-1 strain from studies in Dutch toddlers (2–3 years of age) and school children (7–8 years of age) were used [20]. Briefly these subjects received three vaccinations, the second being given 7 (for toddlers) and 13 (for school children) weeks after the first. The third vaccination was given 5–9 months later for both age cohorts. Two samples were assayed for each subject: a pre-bleed and a sample taken 4–6 weeks post-second vaccination for six toddlers and six school children, 5–9 months post-second vaccination for one toddler and 4–6 weeks post-third vaccination for six toddlers and 11 school children.

Table 1
Phenotypes and VR2 peptide sequences of the meningococcal strains used in the serum bactericidal antibody assay

Lab number	Phenotype	VR2 peptide sequence
M00 242966	B:4:P1.19,15-1	HYTRQNNTDVFVP
M00 242768	B:NT:P1.19-1,15-13	HYTRQNNQNNIDVFVP
M00 243286	B:NT:P1.19-1,15-14	HYTNTRQNNIDVFVP
M00 243089	B:NT:P1.19-12,15-11	HYTRQNNIDVFVP
M01 241666	B:4:P1.19-4,15	HYTRQNNADVFVP

2.2. Serum bactericidal antibody assay

SBA assays were performed as previously described [25] with the minor adaptation of gentle agitation during the serum/complement/bacteria incubation. Human serum, at 25%, was used as an exogenous source of human complement, with titres expressed as the reciprocal of the final dilution giving ≥50% bactericidal killing at 60 min from the control column (active complement/no test sera).

2.3. Strains

The strains used for this study were all wild type isolates collected from cases of meningococcal disease during 2000 and 2001 in the UK (Table 1). Expression of PorA was determined to be comparable for all isolates by SDS-PAGE (data not shown).

2.4. Data analysis

The SBA response was considered as a binary variable with a response of either ≥4, ≥8 or a 4-fold rise. Analyses were performed on the full dataset, then only with those samples where results were obtained for all five strains. For the analysis of responses of ≥4 or ≥8 the data were split into pre- and post-vaccination samples. To allow for the paired nature of the data (i.e, samples with results for more than one strain) conditional logistic regression was used for the analysis. In the analysis, the significance of the variability in responses between strains was tested as well as the interaction between this strain effect and age group.

3. Results

3.1. Compliance with sampling

Due to serum volume constraints, many test sera could not be assayed against all strains. The strains were therefore, prioritised into the following order for assaying: M00 242966 (B:4:P1.19,15-1), M01 241666 (B:4:P1.19-4,15), M00 243089 (B:NT:P1.19-12,15-11), M00 242768