

Induction of immunological memory in UK infants by a meningococcal A/C conjugate vaccine

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SUMMARY

The induction of immunological memory to serogroup A and C polysaccharides in UK infants immunized with three doses of a meningococcal A/C oligosaccharide CRM₁₉₇ conjugate vaccine was investigated. Forty UK infants vaccinated previously with three doses of a meningococcal A/C oligosaccharide-CRM₁₉₇ conjugate vaccine at 2, 3 and 4 months of age, were revaccinated at a mean age of 145·6 weeks with either a 10 or 50 µg dose of licensed meningococcal A/C polysaccharide vaccine. Serogroup-specific antibody and serum bactericidal antibody (SBA) responses were measured by enzyme-linked immunosorbent assay and serum bactericidal assays, respectively. Following challenge, anti-serogroup A and C polysaccharide antibody levels rose from pre-booster geometric mean concentrations (GMC) of 3·1 and 2·1 µg/ml respectively to 19·6 and 21·0 µg/ml 1 month post-booster. Serum bactericidal antibody geometric mean titres (GMTs) for serogroups A and C increased 156- and 113-fold from 2·1 and 7·1 pre-booster respectively to 327·4 and 800·7 post-booster. A serogroup A control group of 45 children received a 10 µg dose of licensed meningococcal A/C polysaccharide vaccine (with no prior history of serogroup A vaccination) had serogroup A SBA GMTs of 2·3 pre-vaccination rising to 8 post-vaccination with corresponding GMCs of 0·8 and 10·8 µg/ml. These rises in SBA following serogroup A/C conjugate vaccination are indicative of immunological priming.

INTRODUCTION

Initial trials addressing the safety and immunogenicity in adults of a bivalent serogroup A and C conjugate vaccine demonstrated significant rises in antibody levels to both A and C polysaccharide and serum bactericidal antibody (SBA) titres to serogroup C meningococci [1]. Serum bactericidal antibodies to serogroup A were not measured in this study [1]. The most important role for an effective meningococcal

conjugate vaccine is the protection of infants and children and generation of long-term immunity.

Studies undertaken in infants in The Gambia with a serogroup A and C polysaccharide-conjugate vaccine demonstrated higher levels of serogroup C antibodies than for vaccination with the native polysaccharide vaccine [2]. Following revaccination with meningococcal A/C polysaccharide vaccine, administered after two doses of a conjugate meningococcal A/C vaccine in Gambian children, immunological memory was demonstrated for serogroup C, but not serogroup A [3].

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Recently, we described a safety and immunogenicity study in UK infants immunized at 2, 3 and 4 months of age with a bivalent meningococcal A/C conjugate vaccine [4]. Immunogenicity was shown by elevated levels of serum anti-serogroup A and C polysaccharide antibodies and SBA activity against two serogroup C isolates [4]. Serum bactericidal activity against serogroup A was not measured in that study [4]. The induction of immunological priming was investigated in this study by measuring the total anti-serogroup A and C immunoglobulin (Ig) and SBA titres following a booster dose of a meningococcal A/C polysaccharide vaccine. The serogroup A SBA data for the primary series of bleeds are also presented. An age-matched control group receiving the licensed meningococcal serogroup A/C polysaccharide vaccine was not included as this was not thought to be ethical due to the induction of immunological hyporesponsiveness to the polysaccharide vaccine [3, 5, 6] which has now been shown to even effect functional antibody responses to subsequent doses of serogroup C conjugate vaccine in healthy adults [7]. However, a control group for serogroup A of infants, with no prior history of serogroup A vaccination, but who received a 10 μg dose of licensed meningococcal serogroup A/C polysaccharide were included. These infants had previously received either one or three doses of a meningococcal serogroup C conjugate vaccine as part of other studies. The SBA titres from a cohort of children were who received a full (50 μg) dose of the meningococcal A/C polysaccharide vaccine as part of an outbreak control measure [8].

MATERIALS AND METHODS

Study population

The study population was as described previously [4] and consisted of 58 infants aged 8–12 weeks who were eligible for routine primary immunization with diphtheria, tetanus, pertussis (DTP) and polio vaccines in the Gloucestershire health district, between January and March 1995. Study nurses obtained written informed consent from parents and participants were immunized according to the standard UK schedule at 2, 3 and 4 months of age. An analogous group of 45 infants, recruited for other vaccine trials, with no prior history of serogroup A vaccination, were also included as controls. Written informed consent was obtained from the parents or guardians of all study subjects and the study protocol was approved by the

West Gloucestershire local research and ethics committee.

Vaccines and immunization

All infants were administered combined Hib oligosaccharide conjugated to diphtheria CRM₁₉₇ (HbOC) (Lederle-Praxis Biologicals, Pearl River, USA) and Evans/Medeva (Leatherhead, UK) DPT whole cell vaccine with 0.6 mg of aluminum hydroxide adjuvant (Al(OH)₃) in the right anterior mid thigh. These vaccines were mixed in one syringe at the time of immunization according to the manufacturers' instructions. Each infant also received oral polio vaccine at each visit.

Infants received the meningococcal vaccine by intramuscular vaccination in the left anterior mid thigh. This included 0.25 ml of meningococcal A/C vaccine (lot 090991/2) and 0.25 ml of Al(OH)₃ (lot 250693) (Biocine Sclavo). Each dose contained 11.2 μg of serogroup A, 11.7 μg of serogroup C oligosaccharide and 48.7 μg of CRM₁₉₇. The production and physicochemical properties of this vaccine have been detailed elsewhere [9].

The initial 10 infants were revaccinated with 0.5 ml (containing 50 μg of both meningococcal A and C polysaccharides) of licensed meningococcal A/C polysaccharide vaccine (AC VAX, SmithKline, Rixensart, Belgium). Following adverse events (described below) in 3 of these infants and in 1 other infant receiving this vaccine in another study, this was changed to a 0.1 ml dose (containing 10 μg of both meningococcal A and C polysaccharides) of an alternative licensed meningococcal A/C polysaccharide vaccine (Mengivac, Pasteur Merieux, Lyon, France).

The serogroup A control group of infants were given a single 10 μg dose of meningococcal A/C polysaccharide vaccine (Mengivac). These infants were selected from two other vaccine trials in which infants had received either a single ($n = 15$) or three ($n = 30$) doses of a meningococcal serogroup C conjugate vaccine (Wyeth Lederle Vaccines and Pediatrics, Pearl River, NY).

Blood samples were obtained by venepuncture from each infant before the first vaccine dose, 4 weeks after each dose of vaccine (including the booster dose) and also at 14 months of age. Sera were sent to the Meningococcal Reference Unit, Manchester Public Health Laboratory, Manchester for analysis.

Laboratory methods

Sera were tested for antibodies to the serogroups A and C capsular polysaccharides by enzyme-linked immunosorbent assay (ELISA) and serum bactericidal assays, against two serogroup C and two serogroup A strains, using standardized protocols as previously reported [4]. ELISA antibody levels in test sera were determined by averaging values for each dilution of serum tested that fell within the working range of the dilution curve of a standard reference serum. The standard reference serum was assigned values of 135.8 $\mu\text{g/ml}$ for serogroup A and 32.0 $\mu\text{g/ml}$ for serogroup C. The conjugate used was a 1:1:1 mixture of anti-human IgG (γ -chain specific), anti-human IgM (μ -chain specific) and IgA (α -chain specific) alkaline phosphatases (Sigma-Aldrich Company Ltd, Poole, Dorset, UK). The antigen was purified native serogroup A or C polysaccharide and methylated human serum albumin. The strains used in the SBA assays were C11 (60E) and G8050 with phenotypes C:16:P1-7^a,1 and C:2a:P1-5,2 respectively and F8238 and L94 4931 both A:4:P1-9. The complement source for all SBA assays was 3- to 4-week-old baby rabbit serum (Pel-Freeze Incorporated, Rodgerson, Arizona, USA). Serum bactericidal antibody titres were expressed as the reciprocal of the final serum dilution giving $\geq 50\%$ killing at 60 min.

Statistical methods

Post-vaccination geometric mean concentrations (GMC) or titres (GMT) with 95% confidence intervals (CI) were calculated for the Ig antibody and SBA levels, respectively. The Mann-Whitney *U* test was used to determine if the differences in antibody levels between different doses were significant.

RESULTS

Study subjects

Forty infants of the original 58 in the study group received three doses of the conjugate vaccine followed by a single dose of the polysaccharide vaccine. The remaining 18 were unavailable for revaccination. The mean age for revaccination was 145.6 weeks (range 105–190). The initial 10 infants received a 50 μg booster dose of polysaccharide vaccine with the subsequent 30 infants receiving a 10 μg dose. Three of the initial 10 infants' sera were insufficient to assay whilst of the subsequent 30 infants, venepuncture failed in 2 infants, 1 infant was withdrawn from the

study and the blood samples from 2 infants were insufficient to assay. For 1 infant the post-booster bleed was collected after 18 weeks therefore this bleed was excluded in the analysis. The mean age for vaccination of the comparative infant group was 82.5 weeks (range 63.1–102.8).

Reactogenicity

No local reactions were seen after the 10 μg booster of meningococcal A/C polysaccharide vaccine but three infants developed adverse events shortly after challenge with a 50 μg dose of the plain polysaccharide vaccine. One subject developed upper lip swelling 3 h post-immunization that resolved spontaneously and had no other symptoms. The second child developed an urticarial rash and facial oedema 3 h post-vaccination who responded to oral antihistamines and settled over 24 h. The third child experienced a transient cough and runny nose post-vaccination and subsequently mild facial erythema, all of which resolved spontaneously. There was no local reaction, fever or any symptoms of anaphylaxis. A full and detailed independent immunological investigation was not able to attribute a specific cause for these reactions.

Serogroup A pre-booster bactericidal titres

No significant difference in SBA titres were observed against the two serogroup A strains used therefore only the titres from isolate F8238 are given. The results of serogroup A SBA GMTs are shown in Table 1. Prior to vaccination 4.3% (2/47) of infants possessed detectable (≥ 8) SBA. A significant increase in bactericidal titre was noted after two doses of conjugate ($P < 0.01$) but no significant difference was seen after one dose or between two and three doses. After one dose of conjugate, 24% (12/50) of infants achieved SBA titres of ≥ 8 , increasing to 76.9% (40/52) and 78.8% (41/52) after two and three doses respectively. Serum bactericidal antibody titres decreased significantly between 5 months of age and 14 months of age ($P < 0.0001$).

Immunogenicity of booster dose

No significant differences were noted between the two different booster doses for Ig or SBA levels against either serogroups A and C, therefore data were combined. The Ig and SBA levels 1 month after revaccination with a meningococcal A/C polysac-

Table 1. Meningococcal serogroup A serum bactericidal antibody titres pre and post three doses of a meningococcal A/C conjugate vaccine and boosting with a dose of meningococcal A/C polysaccharide vaccine

Timing of sample	Serum bactericidal antibody GMT (95% CI)	No. (%) of infants with serum bactericidal titre ≥ 8
Before first dose	2.4 (1.9–2.9)	2/47 (4.3)
After first dose	3.9 (3.0–5.2)	12/50 (24)
After second dose	23.6 (15.5–35.8)	40/52 (76.9)
After third dose	25.2 (16.5–38.5)	41/52 (78.8)
At 14 months	2.1 (2.0–2.3)	0/45 (0)
Post booster	327.4 (193.2–554.8)	30/31 (96.8)

charide vaccine are given in Table 2. Following boosting, 96.8 (30/31) of infants achieved SBA titres of ≥ 8 against serogroups A or C. Serum bactericidal antibody GMTs increased 156- and 113-fold between 14 months of age and 1 month post-booster for serogroups A and C respectively. The 1 infant whose post-booster bleed was collected after 18 weeks and was excluded in the analysis had anti-serogroup A and C polysaccharide levels of 3.5 and 1.6 $\mu\text{g/ml}$ of Ig with corresponding SBA titres of < 4 against both serogroup A and C meningococci. For the control infant group the anti-serogroup A GMCs and serogroup A SBA GMTs (95% CI) were 0.84 $\mu\text{g/ml}$ (0.5–1.4) and 2.3 (1.9–2.9) pre-vaccination rising to 10.8 $\mu\text{g/ml}$ (8.6–13.5) and 8 (5.2–12.5) post-vaccination, respectively. The difference between serogroup A SBA titres in the control and study groups was significant ($P < 0.0001$).

DISCUSSION

Following the successful introduction of the conjugate protein-polysaccharide vaccine for *Haemophilus influenzae* type b, *Neisseria meningitidis* is now the major cause of bacterial meningitis in the United Kingdom [10]. The current licensed meningococcal purified polysaccharide vaccines do not protect children less than 2 years of age from serogroup C disease [11], the age group most at risk of meningococcal disease [10]. We have previously demonstrated that a candidate meningococcal A/C conjugate vaccine was well tolerated and immunogenic when administered at 2, 3 and 4 months of age [4]. In order to investigate whether immunological priming was induced by the conjugate A/C vaccine, the available members of this cohort were challenged with a licensed meningococcal non-conjugated A/C polysaccharide vaccine.

Initially a full 50 μg dose of the polysaccharide

vaccine was administered but with 3 infants, out of the 10 who received this dosage, experiencing adverse reactions the dosage was reduced to 10 μg . No adverse events were then reported. Very high increases of 156- and 113-fold were seen in SBA titres pre to post-booster for serogroups A and C, respectively.

Due to the delay in challenging with purified polysaccharide vaccine, the mean age of the infants was approximately 3 years. At this age infants can mount a protective response to the meningococcal A/C polysaccharide vaccine and indeed, this vaccine is licensed for use in this age group. Therefore it is not clear if this increase in SBA titre was due to immunological priming by the conjugate vaccine or solely a response to the polysaccharide vaccine.

Wilkins and Wehrle (1979) [12] compared 10 and 50 μg dosage of serogroup A polysaccharide vaccine in different age cohorts and found for the 2- to 6-month-old cohort no responders were seen for the 10 μg dose whilst 22.8% responded to the 50 μg dose. This appeared to be an age-dependent effect since in the age cohort 15 months to 4.4 years, 92.3 and 100% responded to the 10 μg and 50 μg doses respectively. Median serogroup A antibody levels for this age group were lower for the 10 μg than the 50 μg dose, 3.4 as oppose to 5.4 $\mu\text{g/ml}$, respectively. Unfortunately SBAs were not performed in this study and to the author's knowledge the SBA of 10 μg dose of polysaccharide vaccine has yet to be reported.

For serogroup A, due to the significant difference ($P < 0.0001$) between SBA GMTs of the serogroup A control infant group (8.0) and the study group (280.2) it appears that the response after polysaccharide revaccination was an anamnestic response rather than a typical T-independent response to the polysaccharide vaccine.

Although minor discrepancies in serum bactericidal methodologies might account for differences, the

Table 2. Meningococcal serogroup A and C specific antibody concentrations before and 1 month after challenge with a dose of meningococcal A/C polysaccharide vaccine in infants who had previously received three doses of the meningococcal serogroup A/C conjugate vaccine at 2, 3 and 4 months of age

	Total anti-serogroup A or C Ig GMC ($\mu\text{g/ml}$) (95% CI)		No. (%) of infants With $> 2 \mu\text{g/ml}$		Serum bactericidal GMT (95% CI)		No. (%) of infants with serum bactericidal titre ≥ 8	
	Serogroup A	Serogroup C	Serogroup A	Serogroup C	Serogroup A	Serogroup C	Serogroup A	Serogroup C
Pre-challenge	3.1 (2.7–3.6)	2.1 (1.7–2.7)	39/47 (83)	28/48 (58.3)	2.1 (2.0–2.3)	7.1 (4.6–11.0)	0/45 (0)	21/46 (45.7)
Post-challenge	19.6 (14.4–26.5)	21.0 (14.3–30.8)	31/31 (100)	31/31 (100)	327.4 (193.2–554.8)	800.7 (430.2–1490.3)	30/31 (96.8)	30/31 (96.8)

findings in a study of 18- to 24-month old US children of SBA GMT of 37.7 following two doses of a 50 μg dose of meningococcal A/C polysaccharide vaccine [13] also lends support to this observation.

This induction of immunological memory is also shown for serogroup C, where serogroup C SBAs, were performed on a cohort of 29 children (median age 178 weeks, range 113–269) who received a full dose of the meningococcal A/C polysaccharide vaccine as part of an outbreak control measure [8]. The SBA GMT for this cohort was 16.4 (95% CI 7.5–35.6) which is significantly lower ($P < 0.0001$) than that of the study group, median age 145.6 weeks (range 105–190) with a SBA GMT of 800.7 (430.2–1490.3). Due to the age of the children receiving a full dose of A/C polysaccharide vaccine and being of a similar (though slightly older) age than the study group who only received a fifth of the dose of A/C polysaccharide, this appears to be strong evidence of an anamnestic response to the conjugate vaccine.

This is further corroborated by the experience with Hib conjugate vaccines that showed induction of immunological memory and long term protection despite a fall in antibody levels [14]. In contrast with our findings, in the Gambian meningococcal A/C conjugate study only the serogroup C component, but not the serogroup A component, appeared to induce immunological memory [3].

At the time of our earlier report, the SBA titres for the primary immunization series had not been determined for serogroup A [4]. Unlike the serogroup C component of the conjugate vaccine, the serogroup A component induced, after a single dose at 2 months of age, a low SBA GMT of only 2.7. Higher levels were achieved after two doses of conjugate vaccine but the SBA titres attained were still considerably lower than those achieved in the Gambian study by the control group who had received three doses of a meningococcal serogroup A purified polysaccharide vaccine [3].

This candidate meningococcal serogroup A/C conjugate is safe, immunogenic and induces immunological memory to both serogroups A and C strains. Meningococcal serogroup A/C conjugate vaccines should induce long term protection from meningococcal serogroup A or C disease and should be considered for introduction into the routine childhood immunization schedule.

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