

## Seroprotection against serogroup C meningococcal disease in adolescents in the United Kingdom: observational study

M D Snape, senior clinical research fellow,<sup>1</sup> D F Kelly, clinical lecturer,<sup>1</sup> S Lewis, research assistant,<sup>1</sup> C Banner, research assistant,<sup>1</sup> L Kibwana, research assistant,<sup>1</sup> C E Moore, post-doctoral research assistant,<sup>1</sup> L Diggle, principal research nurse,<sup>1</sup> T John, senior research nurse,<sup>1</sup> L M Yu, statistician,<sup>2</sup> R Borrow, consultant clinical scientist,<sup>3</sup> A Borkowski, clinical trials director,<sup>4</sup> C Nau, clinical research assistant,<sup>4</sup> A J Pollard, reader in paediatric infection and immunity<sup>1</sup>

<sup>1</sup>Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford OX3 7LJ

<sup>2</sup>Centre for Statistics in Medicine, University of Oxford

<sup>3</sup>Vaccine Evaluation Unit, Health Protection Agency, Manchester

<sup>4</sup>Novartis Vaccines, Marburg, Germany

Correspondence to: M Snape [matthew.snape@paediatrics.ox.ac.uk](mailto:matthew.snape@paediatrics.ox.ac.uk)

doi:10.1136/bmj.39563.545255.AE

### ABSTRACT

**Objective** To determine the persistence of bactericidal antibody titres following immunisation with serogroup C meningococcal glycoconjugate vaccine at age 6-15 years in order to examine changes in persistence of antibodies with age.

**Design** Observational study.

**Setting** Secondary and tertiary educational institutions in the United Kingdom.

**Participants** Healthy adolescents aged 11-20 years previously immunised between 6 and 15 years of age with one of the three serogroup C meningococcal vaccines.

**Intervention** Serum obtained by venepuncture.

**Main outcome measures** Percentage of participants with (rabbit complement) serum bactericidal antibody titres of at least 1:8; geometric mean titres of serogroup C meningococcal serum bactericidal antibody.

**Results** Five years after immunisation, 84.1% (95% confidence interval 81.6% to 86.3%) of 987 participants had a bactericidal antibody titre of at least 1:8. Geometric mean titres of bactericidal antibody were significantly lower in 11-13 year olds (147, 95% confidence interval 115 to 188) than in 14-16 year olds (300, 237 to 380) and 17-20 year olds (360, 252 to 515) ( $P < 0.0001$  for both comparisons). Within these age bands, no significant difference in geometric mean titres of bactericidal antibody between recipients of the different serogroup C meningococcal vaccines was seen. More than 70% of participants had received a vaccine from one manufacturer; in this cohort, geometric mean titres were higher in those immunised at aged 10 years or above than in those immunised before the age of 10.

**Conclusions** Higher concentrations of bactericidal antibody are seen five years after immunisation with serogroup C meningococcal vaccine at age 10 years or above than in younger age groups, possibly owing to immunological maturation. This provides support for adolescent immunisation programmes to generate sustained protection against serogroup C meningococcal disease not only for the vaccine recipients but also, through the maintenance of herd immunity, for younger children.

### INTRODUCTION

In 1999 to 2000 a mass immunisation campaign in the United Kingdom used three different serogroup C meningococcal glycoconjugate vaccines. This campaign aimed to administer a single dose of one of the vaccines to all 1-18 year olds and accompanied the introduction of the serogroup C meningococcal vaccines into the routine infant immunisation schedule. Two of the vaccines contained the serogroup C meningococcal capsular polysaccharide conjugated to a CRM<sub>197</sub> carrier protein (Menjugate, Novartis Vaccines and Diagnostics, Siena, Italy; Meningitec, Wyeth Vaccines, Pearl River, NY, USA), and the third used a tetanus toxoid carrier protein (NeisVac-C, Baxter Vaccines, Beltsville, MD, USA). An uptake of more than 85% was achieved, resulting in a dramatic reduction in serogroup C meningococcal disease.<sup>1</sup>

Enhanced surveillance of serogroup C meningococcal disease and sero-epidemiological studies done after this campaign have shown the importance of maintaining adequate concentrations of specific bactericidal antibody in populations at risk of this disease. Waning effectiveness after immunisation of infants has been associated with a fall in the percentage of children with serum bactericidal antibody titres above the accepted correlates of protection.<sup>2</sup> Children immunised at 2 years of age have shown a similar decline in seroprotection rates,<sup>3</sup> with uncertain effectiveness beyond one year after immunisation.<sup>2</sup> In contrast, sustained elevation of bactericidal antibody titres has been shown three years after immunisation of 9-12 year olds,<sup>4</sup> and this age cohort has no evidence of declining vaccine effectiveness.<sup>2</sup>

No data are available on the persistence of post-immunisation bactericidal antibody titres in children immunised at 6-8 years of age in the UK mass immunisation campaign. Whether this age group will have a sustained increase in their post-immunisation titres of bactericidal antibody against serogroup C meningococcus, like their older counterparts, or whether their immune response will wane like those of younger children is therefore unknown. This lack of

**Table 1 | Recruitment summary**

Event	No
Letters sent	10 949
Replies received	1594
Responded "No"	353
Responded "Yes"	1241
"Yes" responses subsequently excluded	242*
Patients enrolled	999
Patients included in immunogenicity analysis	987†

\*Reasons for exclusion were changed mind to "No"(58), recruitment to study already complete (40), out of age range (2), current antibiotic use (15), recent/planned further immunisation (17), meningococcal serogroup C vaccine naive status reported (47), medical history (17), moved out of area (1), unable to contact (43), and other (2).  
†11 participants did not have history of immunisation with meningococcal serogroup C confirmed; one participant did not have blood drawn.

data is important given that this cohort is now entering adolescence, a period that before the introduction of the vaccines was one of increased risk for serogroup C meningococcal disease.<sup>1</sup> Similarly, no information is available on the persistence of serum bactericidal antibody titres after immunisation of 13-15 year olds, the cohort currently entering young adulthood, or whether the choice of vaccine influences these measures of longer term immunogenicity.

We therefore measured the meningococcal serogroup C specific bactericidal antibody titres and IgG concentrations in blood obtained from 999 adolescents aged 11-20 years who were immunised between the ages of 6 and 15 years. By obtaining the immunisation records of these participants, we determined whether persistence of bactericidal antibody titres was influenced by age of immunisation, specific serogroup C meningococcal immunisation received, or both.

## METHODS

### Study design and participants

The primary objective of this observational study was to evaluate the persistence of specific bactericidal antibody against serogroup C meningococcus in participants aged 11-20 years who received one dose

**Table 2 | Demographics and vaccine received for participants included in immunogenicity analysis. Values are numbers (percentages) unless stated otherwise**

Characteristic	Age band			All ages (n=987)
	11-13 years (n=407)	14-16 years (n=410)	17-20 years (n=170)	
Male	235 (58)	224 (55)	78 (46)	537 (54)
Mean (SD) age (years)	12.8 (0.63)	15.4 (0.85)	18.0 (0.85)	14.8 (2.02)
Mean (SD) age at immunisation (years)	8.1 (0.64)	10.4 (0.77)	12.7 (0.84)	9.9 (1.81)
Mean (SD) time since immunisation (years)	4.7 (0.22)	5.0 (0.30)	5.3 (0.23)	4.9 (0.34)
<b>Vaccine received</b>				
NeisVac-C	65 (16)	31 (8)	0	96 (10)
Menjugate	275 (68)	297 (72)	136 (80)	708 (71)
Meningitec	41 (10)	32 (8)	22 (13)	95 (10)
Unknown	26 (6)	50 (12)	12 (7)	88 (9)

of Menjugate during the 1999 to 2001 vaccination campaign. We did the study in Buckinghamshire and Oxfordshire between March and December 2005. We invited adolescents aged 11-20 attending secondary schools or higher educational establishments to participate, by letter or advertisement. Participants were to be in good health and provide a parental report or self report of having received one of the serogroup C meningococcal vaccines in the UK mass immunisation campaign. Exclusion criteria included immunosuppression, pregnancy, severe medical illness, antibiotic use within 14 days of enrolment, and previous confirmed invasive meningococcal disease. Of the 999 participants to be recruited, at least 100 were to be 14-16 year olds (inclusive) who had been immunised with the serogroup C meningococcal vaccine Menjugate. This cohort was to act as age matched controls for participants recruited in a previous study.<sup>4</sup> For participants aged under 16, consent was given by a parent and the participant; those aged 16 or above gave consent themselves.

After enrolment, we took up to 10 ml of blood and confirmed previous serogroup C meningococcal immunisation by reference to the participant's medical records or the centralised immunisation records of the child health computer departments. We noted details of the age at immunisation and which of the three serogroup C meningococcal vaccines was used when this information was available. In addition, for Menjugate recipients aged 9 and 10 years at serogroup C meningococcal immunisation (14-15 years old at enrolment), we noted details of the receipt of the "school leaver booster" vaccine (combined diphtheria and tetanus vaccine).

### Serological responses

The Vaccine Evaluation Unit, Manchester, analysed serum for bactericidal antibody by using strain C11 (phenotype C:16:P1.7-1,1). Baby rabbit serum (Pel-Freeze, Rodgerson, AZ, USA) was used as an exogenous complement source. Titres of serum bactericidal antibody were expressed as the reciprocal serum dilution yielding 50% or greater killing after 60 minutes. The putative protective rabbit complement serum bactericidal antibody titre (that is, seroprotection) is at least 1:8, but the study protocol specified an additional, conservative, criterion of a rabbit serum bactericidal antibody titre of at least 1:128. We determined the serogroup C meningococcal polysaccharide specific IgG concentration by enzyme linked immunosorbent assays (ELISAs) at Oxford University, using a previously described method.<sup>5</sup> Staff at both laboratories were blinded to the participant's age and the vaccine received.

### Statistical analysis

The analysis of this observational study was descriptive, and we did no formal calculations of study power for the primary objective. We specified a recruitment target of up to 1000 participants in order to provide adequate numbers to obtain information on the

**Table 3** | Markers of immunogenicity for Menjugate recipients. Values in parentheses are 95% confidence intervals

Marker	Age band (age at blood sampling)			
	11-13 years (n=275)	14-16 years (n=297)	17-20 years (n=136)	All ages (n=708)
SBA titre $\geq$ 1:8 (%)	76.4 (70.9 to 81.2)*	87.5 (83.2 to 91.1)	88.2 (81.6 to 93.1)	83.3 (80.4 to 86.0)
SBA titre $\geq$ 1:128 (%)	65.1 (59.1 to 70.7)†	75.1 (69.8 to 79.9)	77.9 (70.0 to 84.6)	71.8 (68.3 to 75.0)
SBA GMT	124 (91 to 170)‡	312 (236 to 413)	346 (232 to 516)	222 (184 to 269)
IgG GMC ( $\mu$ g/ml)	1.1 (0.9 to 1.3)‡	2.6 (2.1 to 3.3)	3.6 (2.6 to 4.9)	1.9 (1.7 to 2.2)

GMC=geometric mean concentration; GMT=geometric mean titre; SBA=serum bactericidal antibody.

\*P=0.001 for comparison with 14-16 year old age band; P=0.007 for comparison with 17-20 year old age band.

†P=0.02 for comparison with 14-16 year old age band; P=0.02 for comparison with 17-20 year old age band.

‡P<0.0001 for comparisons with 14-16 and 17-20 year old age bands.

persistence of seroprotection across all age bands and for all three serogroup C meningococcal vaccines.

We did immunogenicity analyses for Menjugate recipients only and for recipients of the other serogroup C meningococcal vaccines. We analysed immunogenicity data for the whole immunised population and also stratified by age bands based on age at recruitment: 11-13 years, 14-16 years, and 17-20 years.

We calculated geometric mean titres of serogroup C meningococcal bactericidal antibody and geometric mean concentrations of meningococcal serogroup C specific IgG for the above populations, as well as the percentage of participants with rabbit serum bactericidal antibody titres of at least 1:128 and at least 1:8. We also calculated the 95% confidence intervals for these measurements. In an exploratory analysis, we used analysis of variance to compare these measures of immunogenicity between recipients of the different vaccines within the same age band and, for recipients of the same vaccine, within different age bands. We made corrections for the multiple comparisons by the Bonferroni method.

We also explored persistence of antibody after immunisation with Menjugate by calculating the geometric mean bactericidal antibody titres, IgG geometric mean concentrations, and the percentage of Menjugate recipients who were seroprotected, after stratification according to their year of age at immunisation. We used a multiple regression analysis for geometric mean bactericidal antibody titres and geometric mean IgG concentrations to assess the impact of age at immunisation, adjusting for the clustering effect of the school/campus attended, the years since immunisation, and the batch number of Menjugate received and the potential confounding factor of sex. We used multiple logistic regression to evaluate the impact of age at immunisation on the percentage of participants seroprotected, adjusting for the same covariates. For the purposes of analysis by year of immunisation, we pooled the results of those immunised at age 6 and 7 in view of the smaller numbers in these cohorts, as well as those immunised at ages 12-15. We used SPSS version 14.0 and Microsoft Excel to analyse data. We used Stata version 9.2 for multiple regression analysis.

## RESULTS

We recruited a total of 999 participants from 22 educational institutions (median number of

participants per school/campus 13, range 1-174). As outlined in table 1, we evaluated immunogenicity results for 987 participants. The mean age of these participants was 14.8 (SD 2.02) years, and the mean time since immunisation was 4.9 (SD 0.34) years. Table 2 shows further demographic details.

### Antibody persistence in Menjugate recipients according to age at enrolment

Of the 987 participants, 708 (72%) had received Menjugate (mean age 15.0 (SD 2.04) years). Mean time since immunisation was 4.9 years and tended to be slightly longer in older age bands (4.7 years in 11-13 year olds, 5.0 years in 14-16 year olds, and 5.3 years in 17-20 year olds). Serum bactericidal antibody titres of at least 1:8 were shown by 83.3% (95% confidence interval 80.4% to 86.0%) of Menjugate recipients (table 3). When analysed according to age band, the geometric mean bactericidal antibody titres, geometric mean IgG concentrations, and percentage of participants with bactericidal antibody titres of at least 1:8 and at least 1:128 were all significantly lower in 11-13 year old Menjugate recipients than in 14-16 year olds and 17-20 year olds.

### Antibody persistence in Menjugate recipients according to age at immunisation

In order to assess more directly the influence of age on the immune response, we analysed the above measures of immunogenicity according to year of immunisation (fig 1, tables 4 and 5). Whereas the geometric mean bactericidal antibody titres were relatively consistent for participants immunised at age 6-9 years, we saw a marked increase in geometric mean bactericidal antibody titres in those immunised at age 10 (table 4). This was then again relatively consistent for those immunised at older ages. We saw similar, although less marked, trends in seroprotection levels. We also saw a trend to increasing geometric mean serogroup C meningococcal IgG concentrations with increasing age at immunisation (table 4), with a less clear demarcation in antibody concentration between years 9 and 10 than was seen with the geometric mean bactericidal antibody titres.

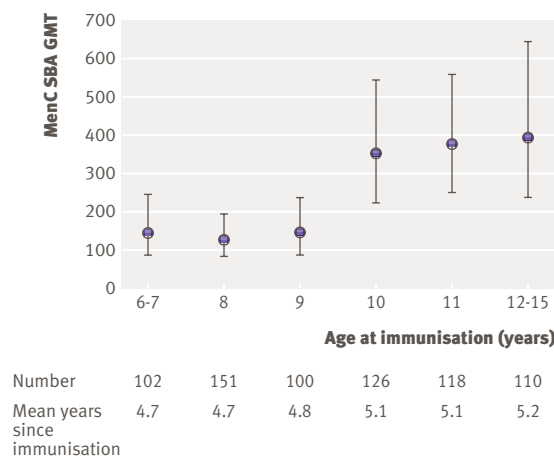
We further analysed this trend by multiple regression analysis, adjusting for sex, time since immunisation, the batch number of Menjugate received, and the clustering effect of school/campus attended. As shown in table 4, even after adjustment for these factors,

**Table 4 | Multiple regression analysis for meningococcal serogroup C specific serum bactericidal antibody geometric mean titres and IgG geometric mean concentrations after immunisation with Menjugate at ages 6-7, 8, 9, 10, and 11 years, compared with immunisation at 12-15 years**

Age at immunisation (years)	Estimated relative difference* (robust 95% CI)	P value
<b>Serum bactericidal antibody geometric mean titres</b>		
Overall	–	<0.001
6-7	0.28 (0.12 to 0.66)	0.005
8	0.23 (0.10 to 0.52)	0.001
9	0.27 (0.11 to 0.69)	0.01
10	0.73 (0.32 to 1.71)	0.4
11	0.84 (0.51 to 1.37)	0.5
12-15	Reference	–
<b>IgG geometric mean concentrations</b>		
Overall	–	<0.001
6-7	0.26 (0.14 to 0.47)	<0.001
8	0.42 (0.27 to 0.65)	<0.001
9	0.48 (0.30 to 0.75)	0.002
10	0.70 (0.41 to 1.21)	0.2
11	0.90 (0.62 to 1.29)	0.5
12-15	Reference	–

\*Adjusted for sex, years since immunisation, batch of Menjugate received, and clustering effect of school/campus attended.

participants immunised at age 6-7, 8, or 9 years of age had significantly lower geometric mean bactericidal antibody titres than those immunised at ages 12-15 years. By contrast, we found no significant difference in the geometric mean bactericidal antibody titres between participants immunised at 10 and 11 years and those immunised at 12-15 years, again supporting an increment in serogroup C meningococcal bactericidal antibodies in those immunised with Menjugate at 10 years of age or above, independent of sex, time since immunisation, batch number received, or clustering effects of school/campus attended. We saw the same increment when we analysed the percentage of participants achieving bactericidal



**Fig 1 | Meningococcal serogroup C (MenC) specific serum bactericidal antibody geometric mean titre (SBA GMT) after Menjugate immunisation, by age of immunisation**

antibody titres of at least 1:128 by logistic regression (again correcting as above) (table 5).

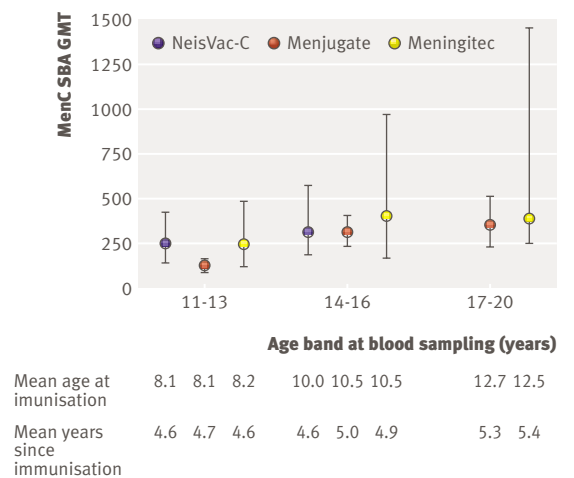
We considered receipt of the “school leaver” combined diphtheria and tetanus vaccine as another possible confounding factor. For those Menjugate recipients for whom we had data, only 20 of 66 aged 9 years at serogroup C meningococcal immunisation had received a booster dose of diphtheria and tetanus vaccine before enrolment, compared with 100 of 108 children aged 10 years at time of immunisation. The close relation between age at immunisation and likelihood of receipt of the school leaver vaccine before enrolment meant that we could not analyse the relation between the receipt of this vaccine and the geometric mean bactericidal antibody titres and geometric mean IgG concentrations independent of age at immunisation.

**Antibody persistence in all serogroup C meningococcal vaccine recipients according to age at enrolment**

We extended analysis of immunogenicity to all 987 participants with confirmed history of receipt of any of the three serogroup C meningococcal vaccines. This showed a meningococcal serogroup C bactericidal antibody titre of at least 1:8 in 830 participants (84.1%, 81.6% to 86.3%). Table 6 shows further measures of immunogenicity for all ages and according to age band (irrespective of vaccine received).

In further analysis for impact of age at enrolment on these measures in non-Menjugate recipients, we saw a non-significant tendency to lower geometric mean bactericidal antibody titres in 11-13 year olds than in 14-16 year olds and 17-20 year olds for Meningitec recipients and to lower geometric mean bactericidal antibody titres in 11-13 year olds than 14-16 year olds for NeisVac-C recipients (fig 2).

We also analysed the geometric mean titres of serogroup C meningococcal bactericidal antibody and geometric mean concentrations of IgG for each vaccine separately within each age band (figs 2 and 3).



**Fig 2 | Meningococcal serogroup C (MenC) serum bactericidal antibody geometric mean titre (SBA GMT) after receipt of MenC vaccine, by age band at blood sampling and vaccine received. No participants in 17-20 year age band received NeisVac-C**

**Table 5** | Logistic regression analysis for percentage of participants achieving serum bactericidal antibody titres  $\geq 1:128$  after immunisation with Menjugate at ages 6-7, 8, 9, 10, and 11 years, compared with immunisation at 12-15 years

Age at immunisation (years)	Odds ratio (robust 95% CI)	P value
Overall	–	<0.001
6-7	0.35 (0.16 to 0.73)	0.005
8	0.38 (0.19 to 0.74)	0.005
9	0.34 (0.16 to 0.74)	0.01
10	0.70 (0.41 to 1.20)	0.2
11	0.96 (0.63 to 1.49)	0.9
12-15	Reference	–

\*Adjusted for sex, years since immunisation, batch of Menjugate received, and clustering effect of school/campus attended.

For 11-13 year olds we saw a tendency for Menjugate recipients to have a lower geometric mean bactericidal antibody titres (124, 95% confidence interval 91 to 170) than recipients of NeisVac-C (256, 151 to 433) or Meningitec (243, 120 to 493). However, analysis of the impact of vaccine received on geometric mean titres of serogroup C meningococcal bactericidal antibody within this age group revealed no significant differences ( $P=0.1$  for Menjugate compared with NeisVac-C and  $P=0.3$  for Menjugate compared with Meningitec). This trend was less obvious when we compared the percentage of participants with bactericidal antibody titres of at least 1:128 (fig 4) or those with bactericidal antibody titres of at least 1:8 (data not shown) or geometric mean IgG concentrations (fig 3).

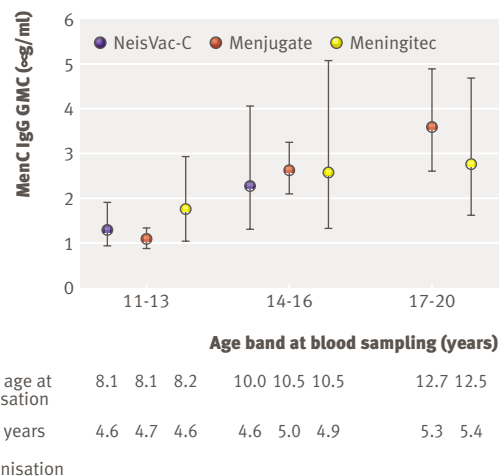
## DISCUSSION

### Key findings

The data presented here show that protective concentrations of antibody persist better after immunisation with a serogroup C meningococcal vaccine in the second decade of life than in the first decade. Whereas most of the 11-20 year olds in this study retained meningococcal serogroup C bactericidal antibody titres of at least 1:128 at a mean of 4.9 years after immunisation, approximately 10% more achieved this correlate of protection in the 14-16 and 17-20 year old Menjugate recipient cohorts than in the 11-13 year olds. Geometric mean titres of bactericidal antibody among Menjugate recipients were similarly higher in the older age groups. These trends were also apparent

**Table 6** | Percentage with serogroup C meningococcal specific serum bactericidal antibody (SBA) titres  $\geq 1:8$  and  $\geq 1:128$ , SBA geometric mean titres (GMT), and IgG geometric mean concentrations (GMCs) of all participants included in immunogenicity analysis (regardless of serogroup C meningococcal vaccine received). Values in parentheses are 95% confidence intervals

	Age band (age at blood sampling)			
	11-13 years (n=407)	14-16 years (n=410)	17-20 years (n=170)	All ages (n=987)
SBA $\geq 1:8$ (%)	79.1 (75.1 to 83.0)	87.3 (84.1 to 90.5)	88.2 (83.4 to 93.0)	84.1 (81.6 to 86.3)
SBA $\geq 1:128$ (%)	68.0 (63.3 to 72.6)	75.4 (70.9 to 79.5)	79.4 (72.5 to 85.2)	73.0 (70.2 to 75.8)
SBA GMT	147 (115 to 188)	300 (237 to 380)	360 (252 to 515)	231 (198 to 270)
IgG GMC ( $\mu\text{g/ml}$ )	1.2 (1.0 to 1.4)	2.5 (2.1 to 3.0)	3.6 (2.7 to 4.6)	2.0 (1.7 to 2.2)



**Fig 3** | Meningococcal serogroup C (MenC) specific IgG geometric mean concentration (GMC) after receipt of MenC vaccine, by age band at blood sampling and vaccine received. No participants in 17-20 year age band received NeisVac-C

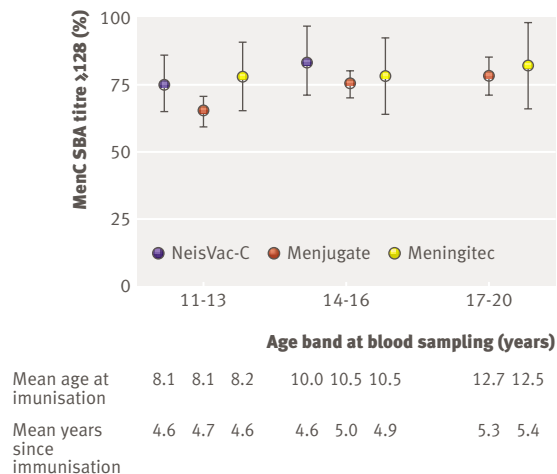
for geometric mean titres of bactericidal antibody and IgG concentrations after receipt of the other serogroup C meningococcal vaccines but did not reach statistical significance (possibly owing to smaller sample sizes).

Previous studies have shown that one year after a primary course of meningococcal serogroup C vaccine at 2, 3, and 4 months of age only 46% of children had serum bactericidal antibody titres of at least 1:8; this had fallen to 12% four years after immunisation.<sup>6</sup> Similarly, after immunisation at age 1-3 years bactericidal antibody titres of at least 1:8 were seen in the serum of only 27% of vaccinees two years after immunisation and only 25% three years after immunisation.<sup>37</sup> By contrast, in this study 75.5% of the children immunised at age 6-7 years had serum bactericidal antibody titres of at least 1:8 at a mean period of 4.7 years after immunisation. In addition, we have described for the first time an increment in the persistence of bactericidal activity that is present among participants immunised from 10 years of age. Increasing age of immunisation beyond 10 years, however, seems to make little further difference to the bactericidal activity seen.

The duration of seroprotection in the years after serogroup C meningococcal immunisation is particularly relevant given that persistence of bactericidal antibody, rather than the anamnestic response to subsequent antigen challenge, is likely to be of primary importance in determining the long term effectiveness of meningococcal serogroup C vaccines.<sup>28</sup> A similar dependence on antibody persistence has been shown for the *Haemophilus influenzae* type b glycoconjugate vaccines,<sup>9</sup> suggesting that this is likely to be relevant for all the glycoconjugate vaccines.

### Explanations for findings

The age dependent variations in bactericidal antibody concentrations that we have described here may be due to participants in the older age groups having had a



**Fig 4 |** Percentage of participants with serum bactericidal antibody (SBA) titres  $\geq 1:128$  after meningococcal serogroup C (MenC) immunisation, by age band and vaccine received. No participants in 17-20 year age band received NeisVac-C

greater response than those in younger age groups to the initial immunisation against serogroup C meningococcus. This was seen in a study in which the response to the three serogroup C meningococcal vaccines was 1.3 to 3.7 times higher in adolescents (mean age 15.1 years) than in younger children (mean age 4.3 years).<sup>10</sup> Alternatively, it may be due to a slower subsequent decline in antibody titre. As outlined below, immunological maturation is a possible explanation for both these phenomena. Alternatively, greater rates of nasopharyngeal carriage of serogroup C meningococcal bacteria (or exposure to cross reactive organisms) may result in greater priming or boosting of the response to the serogroup C meningococcal vaccine in the older age groups. In addition, other, non-immunological, explanations such as systematic biases in our study (for example, an effect of the school attended, independent of the age at vaccine receipt) need to be considered and are also discussed below.

Immunological maturation through early childhood is well described. Suggested mechanisms include the waning of maternal antibody,<sup>11</sup> the developing ability to generate germinal centres after antigenic stimulation (in turn influencing the initial B cell and antibody response),<sup>12</sup> and increased availability of bone marrow niches for long lived plasma cells (postulated to be relevant to the persistence of specific antibody).<sup>13</sup> Our study suggests the possibility that further, previously unrecognised, maturation of the immune response to the serogroup C meningococcal polysaccharide capsule occurs in the second decade of life.

Another possible explanation for the greater bactericidal antibody concentrations in participants immunised at age 10 or above is that these cohorts have experienced a greater degree of immune priming or boosting through nasopharyngeal carriage of serogroup C meningococcus than those immunised at an earlier age. Most of the children immunised at age 10

would have been entering secondary school within a year after the introduction of the serogroup C meningococcal vaccines. Adolescence is the age of peak nasopharyngeal carriage of serogroup C meningococcus, and, although cross sectional surveillance studies done before the introduction of the serogroup C meningococcal vaccine suggest a carriage rate of only 0.45% in 15-17 year olds,<sup>14</sup> studies in the pre-vaccination era showed this to be the age of peak carriage.<sup>15,16</sup> Also, longitudinal studies might show broader population exposure to the organism. Given the rapid decline in rates of nasopharyngeal carriage of serogroup C meningococcus in the year after the introduction of serogroup C meningococcal vaccine,<sup>14</sup> children immunised at age 10 or above might have had more natural exposure to serogroup C meningococcus than those entering secondary school in subsequent years. This provides an alternative explanation for the transition to higher bactericidal titres in this age group. Immunological priming by exposure to cross reactive antigens such as *Escherichia coli* K92 has also been suggested as a factor in the greater response to meningococcal polysaccharides with increasing age.<sup>4,17</sup>

The existence of better immune responses to the meningococcus in older children is supported by both the UK effectiveness studies of the serogroup C meningococcal glycoconjugate vaccines and Canadian studies of the effectiveness of the plain polysaccharide serogroup C meningococcal vaccines.<sup>2,18</sup> The Canadian studies showed a strong trend to increased effectiveness for children immunised at ages 10-14 (effectiveness 75%, 95% confidence interval -17% to 93%) compared with those immunised at ages 2-9 years (effectiveness 41%, -106% to 79%).

#### Comparisons with previous studies

The influence of maturation from the first to the second decade of life on the immune response to other vaccines varies. After immunisation with a 14-valent pneumococcal plain polysaccharide vaccine, 11-12 year old children tended to generate higher geometric mean concentrations of IgG specific to serotypes 14, 18C, 19F, and 23F than did 8-10 year old children,<sup>19</sup> but this was not replicated in a subsequent study.<sup>20</sup> The antibody response and persistence after immunisation with hepatitis A vaccine were no greater in 9-14 year old children than 4-6 year olds.<sup>21</sup> Likewise, a booster dose of measles, mumps, and rubella vaccine elicited similar responses in 4-6 year olds and 11-13 year olds,<sup>22</sup> whereas responses to varicella immunisation are relatively impaired in adolescence compared with early childhood.<sup>23</sup> These studies suggest that the development of the response to immunisation in the second decade of life depends on the nature of the stimulating antigen as well as age related factors. We are not aware of any studies assessing the maturation of the immune response to other glycoconjugate vaccines in the years around puberty.

**WHAT IS ALREADY KNOWN ON THIS TOPIC**

Waning protection after immunisation with serogroup C meningococcal vaccines in infancy and early childhood has been associated with a fall in serum bactericidal antibody concentrations

By contrast, immunisation of 10-12 years olds results in sustained elevation of antibody concentrations, likely to provide protection through adolescence and young adulthood

The age of childhood at which this alteration in immune persistence occurs is unknown

**WHAT THIS STUDY ADDS**

Serogroup C meningococcus specific antibodies were more persistent five years after immunisation in the second decade of life than in the first decade

When corrected for age at administration, the type of serogroup C meningococcal vaccine received made no significant difference to antibody persistence

**Strengths and weaknesses of the study**

The possibility that the increase in persistence of serogroup C meningococcal bactericidal titres seen in this study in children aged over 10 was due to factors other than immunological maturation or differences in priming/boosting needs consideration. No systematic bias in the collection, handling, or analysis of the blood samples could be identified. The similarity of the trends seen between serum bactericidal activity and IgG assays, done in different laboratories by staff blinded to the ages of participants, suggests that no bias in the laboratory analysis was present. In addition, the increase in bactericidal antibodies seen was independent of sex or clustering due to the school or campus attended, the batch number of Menjugate received, or the time between immunisation and blood sampling. We considered receipt of the school leaver booster diphtheria and tetanus vaccine (routinely administered at approximately 14 years of age in the UK, and therefore more commonly received by those immunised against serogroup C meningococcus five years previously at 10 years of age than those immunised at age 9) as another possible confounding factor. In this respect, we should note that the carrier protein used by Menjugate (CRM<sub>197</sub>) is a mutant of diphtheria toxoid. However, because of the close relation between age at immunisation and receipt of the diphtheria and tetanus booster vaccine, we could not analyse independently the impact of receipt of this vaccine. That said, stimulation of B or T cells specific to the carrier protein would seem unlikely to also result in the stimulation of B cells specific for the conjugated polysaccharide capsule, except as part of a non-specific bystander effect as suggested by some authors.<sup>24</sup> Finally, the lack of a control group who had not received serogroup C meningococcal vaccine raises the possibility that the age dependent differences seen relate to acquisition of natural immunity. However sero-epidemiological studies done before the introduction of the serogroup C meningococcal vaccine in the UK showed serogroup C meningococcal bactericidal antibody titres of at least 1:128 in only 5-10% of 10-19 year olds,<sup>25</sup> suggesting that this is unlikely.

This study was designed to assess the persistence of seroprotection afforded by the serogroup C

meningococcal vaccines and not as a sero-epidemiological study. We therefore aimed to selectively recruit serogroup C meningococcal vaccine recipients, rather than a cohort representative of all 11-20 year olds in the UK. Nevertheless, given that the UK mass immunisation campaign achieved coverage rates of greater than 85% in most targeted cohorts,<sup>1</sup> the figures we report here are unlikely to greatly overestimate seroprotection levels in the community. Most adolescents and young adults in the UK therefore seem to have bactericidal antibody concentrations above even the strictest correlate of protection. However, a significant minority lack seroprotection against serogroup C meningococcal disease; among those aged 11-13 years at the time of this study, this figure is more than 20% and presumably will continue to grow with increased time since immunisation.

**Clinical relevance**

The new routine immunisation schedule introduced in the UK in September 2006 provides a booster dose of vaccine against serogroup C meningococcal disease in the second year of life and is likely to improve persistence of antibodies, but no population data are available to indicate the longer term success of this new strategy. However, we know that the previous primary schedule did not induce persistence of protective concentrations of bactericidal antibody. Over the next five years, a cohort of children with very low levels of serogroup C meningococcal seroprotection, who received primary immunisation in early childhood without a booster dose, will be entering adolescence in the UK. This cohort will be susceptible not only to invasive meningococcal disease but also to nasopharyngeal carriage of serogroup C meningococcus. The herd immunity that is currently indirectly protecting those with low antibody concentrations is therefore likely to wane. Accordingly, we suggest that a booster dose of serogroup C meningococcal vaccine administered in early adolescence would be appropriate for this cohort. On the basis of this study, we can be confident that this booster dose would maintain high concentrations of bactericidal antibodies through adolescence and into early adulthood, regardless of which serogroup C meningococcal vaccine is used. This should in turn provide protection not only to the vaccine recipients but also, through maintenance of herd immunity, to the younger children who have not received booster doses and among whom direct vaccine effectiveness is negligible.

We acknowledge the contributions of Gillian Milne and Sally Bedlow, who were responsible for enrolling and sampling most of the participants in this study, and Emma Godfrey, who collected participants' immunisation history. We also thank the participants of this study and the educational institutions that facilitated this research.

**Contributors:** MDS was involved in the development of the study protocol and conduct of the study, was primarily responsible for analysis of the data (having had full access to the data), prepared the first draft of the manuscript, and is the guarantor. DFK was involved in the study design and had significant intellectual input into the preparation of the manuscript. SL, CB, LK, CM, and RB were responsible for the laboratory analysis of the study samples. LD and TJ were involved in the study design and the conduct and supervision of the study. LMY provided significant intellectual

input into the statistical analysis of the study data and preparation of the manuscript. AB and CN were involved in the development of the protocol. AJP proposed the study, was principal investigator of the study, was involved in development of the study protocol, and had significant intellectual input into the analysis of the data and preparation of the manuscript. All authors approved the final version of the manuscript.

**Funding:** This study was funded by Novartis Vaccines and Diagnostic (previously Chiron Vaccines). With the exception of the authors employed by Novartis Vaccines (AB and CN), no authors have received direct payment from Novartis Vaccines. The sponsor funded the study and developed the study protocol in collaboration with AJP, MDS, and DFK. Employees of the sponsor reviewed the manuscript before submission for publication. MDS and TJ are funded by the Oxford Partnership Comprehensive Biomedical Research Centre with funding from the NIHR Biomedical Research Centre Programme, which also provides support to the Oxford Vaccine Group. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health. AJP is a Jenner Institute Investigator.

**Competing interests:** MDS has received financial assistance from Wyeth Vaccines and Novartis Vaccines to attend conferences and has had travel and accommodation expenses paid by Novartis Vaccines while working in collaboration with Novartis Vaccines in Siena, Italy. AJP acts as chief investigator for clinical trials conducted on behalf of Oxford University, sponsored by vaccine manufacturers (Novartis Vaccines, GlaxoSmithKline, Sanofi-Aventis, Sanofi-Pasteur MSD, and Wyeth Vaccines), and has received assistance from vaccine manufacturers to attend scientific meetings. Industry sourced honorariums for lecturing or writing are paid directly to an independent charity or an educational/administrative fund held by the Department of Paediatrics, University of Oxford. AB and CN are employees of Novartis Vaccines.

**Ethical approval:** Ethical approval was granted by Oxfordshire Research Ethics Committee A (approval number CO2.328).

**Provenance and peer review:** Not commissioned; externally peer reviewed.

- 1 Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. *Vaccine* 2001;20(suppl 1):S58-67.
- 2 Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet* 2004;364:365-7.
- 3 Snape MD, Kelly DF, Green B, Moxon ER, Borrow R, Pollard AJ. Lack of serum bactericidal activity in preschool children 2 years after a single dose of serogroup C meningococcal polysaccharide-protein conjugate vaccine. *Pediatr Infect Dis J* 2005;24:128-31.
- 4 Snape MD, Kelly DF, Salt P, Green S, Snowden C, Diggle L, et al. Serogroup C meningococcal glycoconjugate vaccine in adolescents: persistence of bactericidal antibodies and kinetics of the immune response to a booster vaccine more than 3 years after immunization. *Clin Infect Dis* 2006;43:1387-94.
- 5 Gheesling LL, Carlone GM, Pais LB, Holder PF, Maslanka SE, Plikaytis BD, et al. Multicenter comparison of Neisseria meningitidis serogroup C anti-capsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. *J Clin Microbiol* 1994;32:1475-82.
- 6 Borrow R, Goldblatt D, Andrews N, Southern J, Ashton L, Deane S, et al. Antibody persistence and immunological memory at age 4 years after meningococcal group C conjugate vaccination in children in the United Kingdom. *J Infect Dis* 2002;186:1353-7.
- 7 McVernon J, MacLennan J, Buttery J, Oster P, Danzig L, Moxon ER. Safety and immunogenicity of meningococcus serogroup C conjugate vaccine administered as a primary or booster vaccination to healthy four-year-old children. *Pediatr Infect Dis J* 2002;21:747-53.
- 8 Auckland C, Gray S, Borrow R, Andrews N, Goldblatt D, Ramsay M, et al. Clinical and immunologic risk factors for meningococcal C conjugate vaccine failure in the United Kingdom. *J Infect Dis* 2006;194:1745-52.
- 9 McVernon J, MacLennan J, Pollard AJ, Oster P, Wakefield MJ, Danzig L, et al. Immunologic memory with no detectable bactericidal antibody response to a first dose of meningococcal serogroup C conjugate vaccine at four years. *Pediatr Infect Dis J* 2003;22:659-61.
- 10 Burrage M, Robinson A, Borrow R, Andrews N, Southern J, Findlow J, et al. Effect of vaccination with carrier protein on response to meningococcal C conjugate vaccines and value of different immunoassays as predictors of protection. *Infect Immun* 2002;70:4946-54.
- 11 Siegrist CA. Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants. *Vaccine* 2003;21:3406-12.
- 12 Pihlgren M, Tougne C, Bozzotti P, Fulurija A, Duchosal MA, Lambert PH, et al. Unresponsiveness to lymphoid-mediated signals at the neonatal follicular dendritic cell precursor level contributes to delayed germinal center induction and limitations of neonatal antibody responses to T-dependent antigens. *J Immunol* 2003;170:2824-32.
- 13 Pihlgren M, Friedli M, Tougne C, Rochat AF, Lambert PH, Siegrist CA. Reduced ability of neonatal and early-life bone marrow stromal cells to support plasmablast survival. *J Immunol* 2006;176:165-72.
- 14 Maiden MC, Stuart JM. Carriage of serogroup C meningococci 1 year after meningococcal C conjugate polysaccharide vaccination. *Lancet* 2002;359:1829-31.
- 15 Cartwright KA, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and Neisseria lactamica. *Epidemiol Infect* 1987;99:591-601.
- 16 Caugant DA, Hoiby EA, Magnus P, Scheel O, Hoel T, Bjune G, et al. Asymptomatic carriage of Neisseria meningitidis in a randomly sampled population. *J Clin Microbiol* 1994;32:323-30.
- 17 Glode MP, Robbins JB, Liu TY, Gotschlich EC, Orskov I, Orskov F. Cross-antigenicity and immunogenicity between capsular polysaccharides of group C Neisseria meningitidis and of Escherichia coli K92. *J Infect Dis* 1977;135:94-104.
- 18 De Wals P, De Serres G, Niyonsenga T. Effectiveness of a mass immunization campaign against serogroup C meningococcal disease in Quebec. *JAMA* 2001;285:177-81.
- 19 Paton JC, Toogood IR, Cockington RA, Hansman D. Antibody response to pneumococcal vaccine in children aged 5 to 15 years. *Am J Dis Child* 1986;140:135-8.
- 20 Sorensen RU, Leiva LE, Javier FC 3rd, Sacerdote DM, Bradford N, Butler B, et al. Influence of age on the response to Streptococcus pneumoniae vaccine in patients with recurrent infections and normal immunoglobulin concentrations. *J Allergy Clin Immunol* 1998;102:215-21.
- 21 Castillo de Febres O, Chacon de Petrola M, Casanova de Escalona L, Naveda O, Naveda M, Estopinan M, et al. Safety, immunogenicity and antibody persistence of an inactivated hepatitis A vaccine in 4 to 15 year old children. *Vaccine* 1999;18:656-64.
- 22 Johnson CE, Kumar ML, Whitwell JK, Staehle BO, Rome LP, Dinakar C, et al. Antibody persistence after primary measles-mumps-rubella vaccine and response to a second dose given at four to six vs. eleven to thirteen years. *Pediatr Infect Dis J* 1996;15:687-92.
- 23 Hambleton S, Gershon AA. Preventing varicella-zoster disease. *Clin Microbiol Rev* 2005;18:70-80.
- 24 Traggiai E, Puzone R, Lanzavecchia A. Antigen dependent and independent mechanisms that sustain serum antibody levels. *Vaccine* 2003;21(suppl 2):S35-7.
- 25 Trotter C, Borrow R, Andrews N, Miller E. Seroprevalence of meningococcal serogroup C bactericidal antibody in England and Wales in the pre-vaccination era. *Vaccine* 2003;21:1094-8.

Accepted: 16 April 2008