Impact of Meningococcal Serogroup C Conjugate Vaccines on Carriage and Herd Immunity

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(See the editorial commentary by Stephens, on pages 643–5.)

Background. In 1999, meningococcal serogroup C conjugate (MCC) vaccines were introduced in the United Kingdom for those under 19 years of age. The impact of this intervention on asymptomatic carriage of meningococci was investigated to establish whether serogroup replacement or protection by herd immunity occurred.

Methods. Multicenter surveys of carriage were conducted during vaccine introduction and on 2 successive years, resulting in a total of 48,309 samples, from which 8599 meningococci were isolated and characterized by genotyping and phenotyping.

Results. A reduction in serogroup C carriage (rate ratio, 0.19) was observed that lasted at least 2 years with no evidence of serogroup replacement. Vaccine efficacy against carriage was 75%, and vaccination had a disproportionate impact on the carriage of sequence type (ST)-11 complex serogroup C meningococci that (rate ratio, 0.06); these meningococci also exhibited high rates of capsule expression.

Conclusions. The impact of vaccination with MCC vaccine on the prevalence of carriage of group C meningococci was consistent with herd immunity. The high impact on the carriage of ST-11 complex serogroup C could be attributed to high levels of capsule expression. High vaccine efficacy against disease in young children, who were not protected long-term by the schedule initially used, is attributed to the high vaccine efficacy against carriage in older age groups.

Bacterial meningitis and septicemia are among the diseases most feared by parents of young children worldwide. An important causative organism, *Neisseria meningitidis*, is a frequent commensal in the human nasopharynx, with a population carriage rate of around 10% in industrialized countries [1–3]. Meningococcal serogroup C disease has been endemic in the United Kingdom for at least 30 years, but a steady rise in the number of serogroup C meningococcal infections among adolescents during the 1990s [4] prompted the United Kingdom Departments of Health to implement the first national vaccination program with meningococcal serogroup C conjugate (MCC) vaccine. Infants received 2 doses at age 2 months, 3 months, and 4 months, and older children and teenagers up to the age of

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19 years were offered a single dose; vaccinations for those aged 5 years and over were conducted through schools [5]. Clinical trials assuring the safety and immunogenicity of the vaccine had been carried out, and the vaccine was licensed on the basis of serological correlates of protection [6] without direct evidence of efficacy. As a consequence, enhanced surveillance was established to monitor any changes in disease trends following introduction of the vaccine [7, 8].

The high genetic and antigenic diversity of meningococcal populations is relevant to disease incidence; outbreaks of disease caused by this normally harmless bacterium indicate the presence in a human population of hyperinvasive meningococci that express one of the disease-associated capsules, corresponding to serogroups A, B, C, Y, W-135, or, more rarely, X. Multilocus sequence typing (MLST) [9] has demonstrated that most meningococcal disease in Europe is caused by meningococci that belong to the sequence type (ST)-8, ST-11, ST-32, ST-41/44, or ST-269 clonal complexes [10]. The increased incidence of serogroup C disease in the United Kingdom during the 1990s was the result of the spread of a serogoup C variant of the ST-11 complex (formerly ET-37 complex [11]), first identified in Canada [12].

The impact of the United Kingdom MCC vaccine program on meningococci carried by individuals aged 15–19 years in the United Kingdom was investigated. The study was powered to detect changes in the population of serogroup C ST-11 complex bacteria [13], which, although rarely isolated from carriers, are responsible for a disproportionate number of cases of invasive disease [14]. Changes in the prevalence of all clonal complexes and the proportion of meningococci expressing each of the disease-associated serogroups were also investigated. Data on the effect of vaccination 1 year after its introduction on the carriage of meningococci that were phenotypically serogroup C have been published as a research letter [13]. The definitive results of the 2-year studies showing the impact of the MMC vaccine program on carriage of meningococcal genotypes are presented here for the first time.

METHODS

Study design. The study comprised 3 consecutive cross-sectional surveys of meningococcal carriage in 15–19-year-old students attending school or college full or part time (i.e., in further education but not attending a university). The surveys were conducted during vaccine introduction (November and December 1999) and after vaccine introduction (November and December 2000 and 2001). Subjects were recruited in 8 geographical regions throughout the United Kingdom: Bangor, Cardiff, Glasgow, London, Nottingham, Oxford, Plymouth and Stockport, as described elsewhere [13, 15]. For the later surveys, all subjects had been offered vaccination in 1999, and a small number of subjects were sampled on successive years. The age group of the study subjects was chosen because this group was the first to be vaccinated, it was readily accessible, and it exhibits high rates of meningococcal carriage [3]. The Trent Multi-Centre Research Ethics Committee approved the study.

Sample population and sampling methods. Nasopharyngeal swab samples were obtained through the mouth using cotton swabs, which were either plated on site or placed in transport medium to be plated in the laboratory within 5 hours. Samples were plated on selective plate medium that contained gonococcal agar base, antibiotics (vancomycin, colistin, nystatin, and trimethoprim), and 5% lysed or whole horse blood. The inoculated plates were incubated at 37°C in an atmosphere of 5% carbon dioxide and visually inspected for putative *Neisseria* colonies at 24 and 48 hours. Putative *Neisseria* colonies were examined by Gram film (gram-negative diplococci) and examined for oxidase-positive reaction. Individual colonies were subcultured to pure culture. Where necessary, these were further discriminated by Gonocheck (EY) prior to storage in glycerol broth or with a commercial bead system (Microbank; Prolab) at −20°C to −80°C. Cultures that were positive for *N. meningitidis* were used to prepare lysed cell suspensions by sweeping 10–20 colonies and emulsifying them in 2 mL of saline. A sample (250 μL) of this suspension was placed in a labeled tube and heated to 100°C for 5 min. Tubes were then snap-chilled at −20°C for 5 min, and centrifuged at 20,000 *g* for 10 min. Meningococcal cultures were not available for the London center for 1999, and this center was not included in the analysis.

Isolate characterization. MLST and *siaD* gene amplification and nucleotide sequencing were performed in accordance with published methods [16–19], employing robotic liquid handling and automated DNA analyzers. MLST determines the nucleotide sequences of 7 housekeeping gene fragments, each of which is assigned a unique allele number; the combination of the 7 numbers represents an ST. The STs were assigned to clonal complexes [11] by reference to the *Neisseria* pubMLST database (http://pubmlst.org/neisseria). The presence of capsule-specific genes was determined by polymerase chain reaction (PCR) amplification of the *siaD* gene or the capsule null locus *(cnl)* in the capsular region. Meningococci with the *cnl* locus are unable to synthesize a capsule. Where present, the nucleotide sequence of the *siaD* gene was determined, to establish the presence of alleles encoding the polysialyltransferases that synthesize serogroup B, C, Y, or W-135 capsules.

Data manipulation and statistical analysis. Sequences were assembled using the STARS (Sequence Typing Analysis and Retrieval System) [20] package, which is integrated into the Staden package [21] (available from http://pubmlst.org/software). Data on isolates were stored in a proprietary database based on the mlstdbNet Software [22]. Statistical analyses were performed using Intercooled Stata software for windows (version 8.0; StataCorp). Proportions were compared using a χ^2 test or Fisher exact test, as appropriate. Data are presented for the 3 years of the study. All comparisons over time were made between 1999 and 2001. There was a small degree of overlap between individ-

Table 1. Carriage of meningococci encoding disease-associated capsules and their serogroup.

Capsular genotype or phenotype	Meningococci, % (proportion) of isolates			Rate ratio 2001:1999 (95% CI)	*P*
	1999	2000	2001		
siaD$_C$	5.88 (138/2348)	3.10 (91/2931)	2.68 (89/3320)	0.46 (0.35–0.59)	<.001
Serogroup C	2.51 (59/2348)	0.72 (21/2931)	0.48 (16/3320)	0.19 (0.11–0.33)	<.001
siaD$_B$	33.86 (795/2348)	34.22 (1003/2931)	35.96 (1185/3320)	1.05 (0.98–1.13)	.15
Serogroup B	23.00 (540/2348)	22.76 (667/2931)	24.10 (800/3320)	1.05 (0.95–1.15)	.34
siaD$_W$	10.14 (238/2348)	13.03 (382/2931)	12.32 (409/3320)	1.21 (1.05–1.41)	.01
Serogroup W	6.30 (148/2348)	7.51 (220/2931)	7.14 (237/3320)	1.13 (0.93–1.38)	.22
siaD$_Y$	9.97 (234/2348)	10.13 (297/2931)	9.73 (323/3320)	0.98 (0.83–1.15)	.77
Serogroup Y	5.58 (131/2348)	5.53 (162/2931)	5.39 (179/3320)	0.97 (0.78–1.20)	.76

NOTE. CI, confidence interval.

uals who were sampled more than once (367 of the 14,056 individuals sampled in 1999 were among the 17,770 individuals sampled in 2001 [2.1% of the 2001 sample]).

RESULTS

In November 1999, 14,057 students were sampled at the same time as vaccine administration; of these, 2348 samples yielded meningococci, a carriage prevalence of 16.7%. In November 2000 and 2001—1 and 2 years after vaccine introduction—the prevalence of carriage was 17.7% (12,931 of 16,482) and 18.7% (3320 of 17,770) respectively. The increase in overall carriage prevalence over these 3 years (rate ratio 2001:1999, 1.12) was statistically significant ($P < .001$), but varied among sampling centers (rate ratios, 0.85–1.59; data not shown).

From 1999 to 2001, there was a large decrease in the prevalence of carriage of meningococci that expressed serogroup C and also in carriage of meningococci that contained the *siaD*$_C$ gene responsible for C capsule production (rate ratio, 0.19 and 0.46, respectively; $P < .001$ for both) (table 1). The prevalence of carriage of meningococci that expressed other serogroups increased slightly or remained similar over the duration of the study (rate ratios, 0.97– 1.21; table 1). A logistic regression model showed no evidence of differences between centers with respect to trends in the prevalence of carriage by serogroup, apart from serogroup Y, which decreased in some centers and increased in others. Analysis by clonal complex, *siaD*$_C$ gene, and serogroup expression confirmed a relatively low prevalence of carriage of ST-11 complex *siaD*$_C$ meningococci—the predominant epidemic strain—in 1999 (tables 1 and 2). The prevalence of carriage of this clonal complex fell sharply by 83% in 2000 and 94% in 2001 ($P < .001$) (table 2). There was some evidence for reduction in the prevalence of carriage of the other disease-associated meningococcal genotypes with the *siaD*$_C$ gene, but

Table 2. Carriage of meningococci by clonal complex and capsule-specific genes.

Meningococcal genotype	Meningococci, % (proportion) of isolates			Rate ratio 2001:1999 (95% CI)	*P*
	1999	2000	2001		
ST-11					
siaD$_C$	1.83 (43/2348)	0.78 (23/2931)	0.21 (7/3320)	0.11 (0.05–0.25)	<.001
Serogroup C	1.49 (35/2348)	0.31 (9/2931)	0.09 (3/3320)	0.06 (0.02–0.19)	<.001
ST-8					
siaD$_C$	0.26 (6/2348)	0.03 (1/2931)	0.09 (3/3320)	0.35 (0.09–1.41)	.18
Serogroup C	0.13 (3/2348)	0.03 (1/2931)	0.06(2/3320)	0.47 (0.08–2.82)	.66
ST-41/44					
siaD$_C$	1.19 (28/2348)	0.61 (18/2931)	0.54 (18/3320)	0.45 (0.25–0.82)	.007
Serogroup C	0.38 (9/2348)	0.10 (3/2931)	0.09 (3/3320)	0.24 (0.06–0.87)	.035
siaD$_B$	12.95 (304/2348)	11.91 (349/2931)	11.63 (386/3320)	0.90 (0.78–1.03)	.13
Serogroup B	8.90 (209/2348)	8.39 (246/2931)	8.10 (269/3320)	0.91 (0.77–1.08)	.29
Other genotypes *siaD*$_C$	2.60 (61/2348)	1.67 (49/2931)	1.84 (61/3320)	0.71 (0.50–1.00)	.052
Other genotypes serogroup C	0.51 (12/2348)	0.27 (8/2931)	0.24 (8/3320)	0.47 (0.19–1.15)	.091

NOTE. CI, confidence interval.

Table 3. Expression of capsules by carried meningococci.

	Meningococci expressing capsule, % (proportion)			Rate ratio 2001:1999 (95% CI)	*P*
	1999	2000	2001		
Serogroup C/*siaD*$_C$					
All	42.8 (59/138)	23.1 (21/91)	18.0 (16/89)	0.42 (0.26–0.68)	<.001
ST-11	81.4 (35/43)	39.1 (9/23)	42.9 (3/7)	0.53 (0.30–0.93)	.048
ST-8	50.0 (3/6)	100.0 (1/1)	66.7 (2/3)	1.33 (0.43–4.13)	.99
ST-41/44	32.1 (9/28)	16.7 (3/18)	16.7 (3/18)	0.52 (0.16–1.66)	.32
Other	19.7 (12/61)	16.3 (8/49)	13.1 (8/61)	0.67 (0.29–1.52)	.33
Serogroup B/*siaD*$_B$					
All	67.9 (540/795)	66.5 (667/1003)	67.5 (800/1185)	0.99 (0.93–1.06)	.85
ST-41/44	68.8 (209/304)	70.5 (246/349)	69.7 (269/386)	1.01 (0.92–1.12)	.79
All serogroup W/*siaD*$_W$	62.1 (148/238)	57.6 (220/382)	57.9 (237/409)	0.93 (0.82–1.06)	.29
All serogroup Y/*siaD*$_Y$	56.4 (132/234)	54.9 (162/295)	54.7 (179/327)	0.97 (0.84–1.13)	.69

NOTE. CI, confidence interval.

not in prevalence of carriage of the genotypes with the *siaD*$_B$, *siaD*$_Y$, or *siaD*$_W$ genes.

The presence of genes determining particular serogroups and their expression varied with clonal complex (table 3). In 1999, before vaccine introduction, the proportion of *siaD*$_C$ meningococci that expressed their capsule was significantly lower than the proportion of *siaD*$_B$, *siaD*$_W$, and *siaD*$_Y$ meningococci that expressed their capsule. ($P < .01$, $<.001$, and .011, respectively). For *siaD*$_C$ meningococci, capsule expression varied by the clonal complex; most ST-11 *siaD*$_C$ meningococci expressed their capsules (35 of 43 [81%]), whereas the proportion of other clonal complexes with the *siaD*$_C$ gene that expressed their capsule was lower (24 of 95 [25%]; $P < .001$). For *siaD*$_C$ meningococci, there was a reduction in the proportion of meningococci that expressed their capsule from 1999 to 2001 (rate ratio, 0.42 [95% confidence interval {CI}, 0.26–0.68]) (table 3). No change was observed in levels of capsule expression among *siaD*$_B$, *siaD*$_W$, or *siaD*$_Y$ meningococci.

In the 2001 survey, 3235 (97.4%) of the 3320 individuals from whom meningococci were isolated reported their vaccination status; of these, 2986 (92.3%) had been vaccinated. A total of 76 (2.55%) of the vaccinated subjects carried *siaD*$_C$ meningococci, compared with 9 (3.61%) of the 249 unvaccinated subjects; 12 (0.40%) of the vaccinated subjects carried serogroup C meningococci, compared with 4 (1.61%) of the unvaccinated individuals. Vaccine effectiveness against carriage was calculated to be 75% ($100 \times [1-(0.40/1.61)]$; 95% CI, 23%–92%) for serogroup C meningococci and 29% (95% CI, −39% to 64%) for *siaD*$_C$ meningococci. The reduction in expression of serogroup C by *siaD*$_C$ meningococci was not seen among the 17 unvaccinated individuals sampled in 2000 and 2001 who carried *siaD*$_C$ meningococci, 8 (47%) of whom carried meningococci that expressed serogroup C.

DISCUSSION

This large-scale study was designed to account for the anticipated low prevalence of carriage of hyperinvasive ST-11 complex *siaD*$_C$ meningococci. This expectation was confirmed during the course of the study; this complex accounted for only 82 of the 8599 meningococci isolates obtained. Despite these low numbers, however, the study demonstrated that vaccination with MCC vaccine had a statistically significant effect on the prevalence of carriage that was specific to both serogroup and clonal complex. To our knowledge, this study represents the largest survey of meningococcal carriage reported to date. The results demonstrate the feasibility of handling and storage of large sample collections and MLST data sets, the potential application of this technology in large-scale population studies, and the relevance of this approach in studies of vaccine impact.

Over the course of the study, from just before vaccine implementation until 2 years after implementation, there was a small increase in the prevalence of meningococcal carriage (from 16.7% to 18.7%). The most likely explanation for this increase was improved sampling efficiency at some of the centers over the course of the study [23]. The composition of the population of meningococci isolates recovered from carriers remained essentially unchanged, with the exception of the serogroup C, and especially the serogroup C ST-11 complex bacteria, which declined more than any other clonal complex ($P = .005$). By comparison, the prevalence of carriage of the meningococci most commonly associated with serogroup B disease—*siaD*$_B$ members of the ST-41/44 complex—was unaffected by the introduction of vaccine (table 2). These organisms were the second most frequent cause of meningococcal disease in 1999, and they became the most common cause of meningococcal disease after MCC vaccine introduction [4, 24].

The decline in the prevalence of carriage of serogroup C meningococci between 1999 and 2000 that we reported previously [13] was confirmed, and a further decline was evident from 2000 to 2001 (table 1). This result was consistent with herd immunity, i.e., immunity among teenagers that interrupted the transmission of serogroup C meningococci, operating during the introduction of the vaccine [25]. The comparisons of data from vaccinated and unvaccinated individuals provided further evidence of a herd immunity effect. Genotyping of the *siaD* locus showed that the reduction in the prevalence of carriage of serogroup C organisms was the result of 2 effects that were of approximately equal magnitude: (1) the loss of organisms containing the $siaD_C$ allele from the population of carried meningococci and (2) lower rates of expression of the $siaD_C$ gene (tables 1 and 3).

The proportion of $siaD_B$ meningococci that expressed serogroup B capsular polysaccharide was high, as observed in previous studies [26], and this proportion was consistent among clonal complexes, there being no difference between the expression rate of all serogroup B organisms and the expression rate for members of the hyperinvasive ST-41/44 complex. For serogroup C, ST-11 meningococci showed much higher levels of expression (81% of isolates) than $siaD_C$ meningococci not belonging to hyperinvasive lineages (20%). This was consistent with data obtained previously in a carriage study conducted in Bavaria, although the size of the Bavarian study was insufficient for a robust demonstration of this effect [3].

The success of the MCC vaccines in reducing disease in the United Kingdom [5] can therefore be attributed to the combined efficacy of the vaccine against disease and carriage, the high proportion of serogroup C polysaccharide expression among ST-11 complex meningococci, and the nature of the vaccine introduction. Due to a number of high-profile outbreaks in educational establishments prior to vaccine introduction, the 15–19-year-old age group was targeted for vaccination early in the campaign. As this age group is characterized by both high rates of carriage and transmission of meningococci [15], the effects of vaccination on the ST-11 $siaD_C$ meningococci were particularly marked. This was fortuitous given that the infant schedule used for MCC vaccines in the United Kingdom at the time of vaccine introduction did not confer long term protection [27]. Young children were consequently protected from infection by the virtual elimination of the disease-causing meningococci from older age groups (table 3).

It is likely that the susceptibility of ST-11 complex $siaD_C$ meningococci to MCC vaccines was a consequence of their high-level expression of serogroup C polysaccharide, but it is unclear why this should have been so. Although the capsule is the major virulence determinant for meningococci, it cannot have evolved specifically to cause disease because meningococci gain no benefits from causing disease in terms of improved host-to-host transmission [28]. Meningococci that express a disease-associated capsule must, therefore, balance any costs associated with possession of the capsule (in terms of removing hosts from the transmission system) with a benefit, for example, in improved transmission among hosts. The retention of capsule expression during colonization by $siaD_B$ meningococci can be rationalized by the relatively poor immunogenicity of this polysaccharide [29], whereas most $siaD_C$ meningococci downregulate the expression of the more immunogenic serogroup C capsule during carriage. However, it appears from these data that this strategy is not operating in the case of ST-11 complex meningococci, perhaps as a consequence of the capsule having a more important, and incompletely understood, role in the transmission of these low-prevalence bacteria.

Given the very strong effect of the MCC vaccine on ST-11 complex $siaD_C$ meningococci, it is difficult to explain the lack of emergence of ST-11 meningococci expressing other serogroups [8]. Capsular replacement by ST-11 complex serogroup B or W-135 meningococci was a particular concern because these organisms have caused disease outbreaks elsewhere and successional replacements have been reported in various countries [28, 30–32]. It is possible that the relative uniformity of the ST-11 meningococci in their subcapsular antigens [33] was at least partially responsible for this result as a consequence of naturally induced herd immunity against these antigens, but this cannot be concluded from the current data.

Previous experience with conjugate vaccines suggested that protection is not only conferred by the induction of immunological memory among vaccinated individuals [34, 35], but also by reduction of carriage and transmission among the unvaccinated population [36–40]. Indirect protection is a likely explanation for both the immediate repercussions of the mass-vaccination campaign and the persistence and accentuation of the effects over time. Indeed, the campaign's impact on the protection of the unvaccinated is higher by these measures than might have been expected. The prevalence of carriage of the predominant disease-causing meningococcus, ST-11 complex $siaD_C$, showed the most dramatic decline after vaccination, although the vaccine did not specifically target these meningococci. This decline was explained by the relatively high proportion of ST-11 meningococci that expressed C capsule, compared with $siaD_C$ meningococci belonging to other clonal complexes, such that anticapsular antibody generated by vaccination had a disproportionate impact on carriage of the ST-11 complex $siaD_C$ genotype. The strong association of this genotype with the expression of capsule may be linked to its high virulence.

This is the first time that the effects of a national vaccination campaign against bacterial disease have been monitored from its initiation by large-scale carriage studies that have included detailed phenotypic and genetic characterization of the bacterial isolates recovered. Understanding the effects of the United Kingdom's MCC vaccine program on pharyngeal carriage of *N. men-*

ingitidis has and will assist other countries in planning the introduction of conjugate or other novel meningococcal vaccines. In particular, it will be of interest to establish whether the remarkable herd immunity effect of vaccination on the ST-11 $siaD_C$ meningococci, in some ways similar to that observed with Hib vaccines [36], occurs with other lineage and/or serogroup or serotype combinations of encapsulated bacteria. Finally, the data gathered in this study will be invaluable for the development of mathematical models to enhance our understanding of, and to help predict the outcome of, large-scale public health interventions aimed at bacterial populations.

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