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Persistence of serogroup C antibody responses following quadrivalent meningococcal conjugate vaccination in United States military personnel

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\textbf{Abstract}

Serogroup C meningococcal (MenC) disease accounts for one-third of all meningococcal cases and causes meningococcal outbreaks in the U.S. Quadrivalent meningococcal vaccine conjugated to diphtheria toxoid (MenACWY\textsubscript{D}) was recommended in 2005 for adolescents and high risk groups such as military recruits. We evaluated anti-MenC antibody persistence in U.S. military personnel vaccinated with either MenACWY\textsubscript{D} or meningococcal polysaccharide vaccine (MPSV4). Twelve hundred subjects vaccinated with MenACWY\textsubscript{D} from 2006 to 2008 or MPSV4 from 2002 to 2004 were randomly selected from the Defense Medical Surveillance System. Baseline serologic responses to MenC were assessed in all subjects; 100 subjects per vaccine group were tested during one of the following six post-vaccination time-points: 5–7, 11–13, 17–19, 23–25, 29–31, or 35–37 months. Anti-MenC geometric mean titers (GMT) were measured by rabbit complement serum bactericidal assay (rSBA) and geometric mean concentrations (GMC) by enzyme-linked immunosorbent assay (ELISA). Continuous variables were compared using the Wilcoxon rank sum test and the proportion of subjects with an rSBA titer ≥ 8 by chi-square. Pre-vaccination rSBA GMT was < 8 for the MenACWY\textsubscript{D} group, rSBA GMT increased to 703 at 5–7 months post-vaccination and decreased by 94% to 43 at 3 years post-vaccination. GMT was significantly lower in the MenACWY\textsubscript{D} group at 5–7 months post-vaccination compared to the MPSV4 group. The percentage of MenACWY\textsubscript{D} recipients achieving an rSBA titer of ≥ 8 decreased from 87% at 5–7 months to 54% at 3 years. There were no significant differences between vaccine groups in the proportion of subjects with a titer of ≥ 8 at any time-point. GMC for the MenACWY\textsubscript{D} group was 0.14 μg/mL at baseline, 1.07 μg/mL at 5–7 months, and 0.66 μg/mL at 3 years, and significantly lower than the MPSV4 group at all time-points. Anti-MenC responses wane following vaccination with MenACWY\textsubscript{D}; a booster dose is needed to maintain protective levels of circulating antibody.

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\textbf{Abbreviations:} ACIP, Advisory Committee on Immunization Practices; CDC, Centers for Disease Control and Prevention; DMSS, Defense Medical Surveillance System; DoD, Department of Defense; DoDSR, Department of Defense Serum Repository; ELISA, enzyme-linked immunosorbent assay; GMC, geometric mean concentration; GMT, geometric mean titer; LLQ, lower limit of quantitation; MenACWY, quadrivalent meningococcal conjugate vaccine; MenACYW\textsubscript{D}, quadrivalent meningococcal conjugate vaccine conjugated to diphtheria toxoid; MenC, Serogroup C Neisseria meningitidis; MPSV4, quadrivalent meningococcal polysaccharide vaccine; NHRC, Naval Health Research Center; rSBA, rabbit complement serum bactericidal assay; VE, vaccine effectiveness.

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1. Introduction

In the United States, serogroups B, C and Y Neisseria meningitidis each account for approximately one-third of meningococcal cases [1]. From 1998 to 2007, serogroup C (MenC) disease resulted in the highest case fatality ratio (14.6%) among the three serogroups [1]. MenC often results in more severe sequelae in its survivors and has a predilection to cause outbreaks [2–4]. Sequence type (ST) 11/electrophoretic type (ET) 37 clonal complex was responsible for outbreaks in U.S. army military recruits in the 1960s and continues to cause outbreaks in the U.S. today [1,5]. Although disease rates for all serogroups are at a historic low, morbidity and mortality among cases remains unchanged.

Prior to 2005, quadrivalent (A, C, Y, W) meningococcal polysaccharide vaccine, MPSV4 (Menomune®, Sanofi Pasteur, Swiftwater, PA, USA), was used routinely in U.S. military recruits to reduce the risk of disease during basic training. However, routine vaccination of the general population was not recommended because of its limited duration of protection. In 2005, the Advisory Committee on Immunization Practices (ACIP) recommended vaccination of adolescents and other persons at high risk for meningococcal disease with a newly licensed quadrivalent meningococcal conjugate vaccine (MenACYW) [6]. Two quadrivalent meningococcal conjugate vaccines are currently available for adolescents in the U.S. MenACWY® (Menactra®, diphtheria toxoid conjugate, Sanofi Pasteur, Swiftwater, PA, USA) was licensed in 2005 and MenACWY®CRM (Menevaco®CRM, CRM-197 conjugate, Novartis Vaccines, Cambridge, MA, USA) in 2010. The ACIP recommended use of either vaccine for adolescents aged 11–18 years and other persons at increased risk for meningococcal disease, including military recruits and first year college students living in residential housing. Upon licensure, quadrivalent meningococcal conjugate vaccines were expected to provide protection for at least 5–10 years. However, a limited number of persistence studies conducted during clinical trials suggest antibody waning occurs faster than previously predicted [7,8].

To address increasing concern for limited duration of protection following vaccination with meningococcal conjugate vaccine, in January 2011 the ACIP recommended a booster dose for adolescents on or after their 16th birthday to provide optimal protection throughout the period of increased risk (16–21 years of age). Booster doses continue to be recommended every 5 years for high risk groups, such as those with certain immunologic disorders, as well as military personnel who continue to be at increased risk [9]. However, data supporting the optimal interval for vaccination of these high risk groups are limited. The objective of this study is to evaluate antibody persistence to MenC following vaccination with MenACWY® in military personnel to inform US public health policy for quadrivalent meningococcal vaccines. Serologic responses over a 3 year period are compared to military recruits who were routinely vaccinated with meningococcal polysaccharide vaccines (MPSV4) prior to licensure of conjugate vaccines.

2. Materials and methods

2.1. Study design and participants

We conducted a retrospective cohort study among U.S. military service personnel previously vaccinated with either quadrivalent meningococcal conjugate (MenACWY®) or polysaccharide (MPSV4) vaccine. Eligibility criteria included receipt of one dose of MPSV4 from 2002 to 2004 or MenACWY® from 2006 to 2008, availability of sera prior to vaccination, and at least one sample within 3 years post-vaccination. Individuals with a history of ≥2 doses of meningococcal vaccine were excluded. Subjects meeting the eligibility criteria were selected from the U.S. Department of Defense’s (DoD) Defense Medical Surveillance System (DMSS) electronic database. DMSS integrates medical surveillance data for over ten million individuals who have served in the U.S. military since 1990 [10,11]. Sera that had been previously collected and subsequently stored in the Department of Defense Serum Repository (DoDSR) as part of public health surveillance were used to determine serologic responses to meningococcal vaccines.

To determine persistence of antibody responses to MenC following vaccination with MPSV4 or MenACWY®, 1200 subjects, 600 subjects per vaccination group, were randomly selected from DMSS. Basic demographic information, including sex, age and race, and meningococcal vaccination history were obtained from DMSS. Pre-vaccination samples from all subjects were tested to determine baseline levels. Two hundred subjects, 100 subjects per vaccination group, were evaluated during one of six post-vaccination time-points: 5–7 months, 11–13 months, 17–19 months, 23–25 months, 29–31 months, or 35–37 months. Only one post-vaccination sample per subject was tested.

The study was determined exempt from human subjects research review by the Human Subjects Offices at the Naval Health Research Center (NHRC) and Centers for Disease Control and Prevention (CDC).

2.2. Serological responses

Serum bactericidal antibody titers to MenC were measured by a validated rabbit complement serum bactericidal assay (rSBA) using the target strain C11 [12,13]. Viability counts were determined with an automated colony counter (Symbiosis Protocol, United Kingdom). A titer of 1.33 was assigned to sera with no activity in the initial serum dilution of 1:4. Continuous titers were interpolated from 3-fold serum dilutions. Each sample was assigned a continuous titer resulting in ≥50% killing compared to control wells. The proportion of subjects with rSBA titers at or above the putative protective threshold of 8 was calculated [14,15]. A more conservative cutoff of 128 was also used to assess decay of immune responses over time. Serum IgG antiscapular antibody concentrations were determined using a standardized enzyme-linked immunosorbent assay (ELISA) [16]. The concentration of specific IgG antibodies in human sera was calculated relative to a human standard reference serum pool, CDC 1992 [17]. Antibody concentrations below the lower limit of quantitation (LLQ) of 0.001 µg/mL were assigned the LLQ. Data were captured with GenS® (BioTek) and analyzed using ELISA for Windows (CDC, Atlanta, GA). The percent of subjects with IgG antibody concentrations at or above 2 µg/mL was determined [18,19]. Testing was performed blinded to vaccine type and time-point.

2.3. Statistical analysis

Sample size calculations with a two-sided alpha of 0.05 and 80% power were based on previous adult immunogenicity studies of meningococcal conjugate and polysaccharide vaccines to determine the proportion of subjects with a threshold of ≥8 for rSBA titers and ≥2 µg/mL for IgG responses [8,20]. Statistical analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Geometric mean titers (GMT) for rSBA and geometric mean concentrations (GMC) for ELISA were calculated for each vaccine group per time-point. Because the log-transformed data were not normally distributed, continuous variables were compared using the Wilcoxon rank sum test at each time point. A Chi-square test was used to compare categorical variables, including the proportions of subjects with rSBA titers ≥8 and ≥128, as well as the increase in 4-fold response compared to baseline for each time-point. Comparisons with a two-sided P value <0.05 were considered statistically significant. P-values for multiple tests across the seven time points.
were adjusted for multiple comparisons by multiplying tested P-values by 7 and comparing them to 0.05 in a test for significance.

3. Results

Among the 1200 subjects evaluated, 83% were male and 68% were white. The mean age at the time of vaccination was 20.5 years (range, 17–37) for MenACWYD and 20.0 years for MPSV4 (range, 17–34). There were no significant differences in sex and race between vaccine groups. Serum bactericidal activity and antibody concentrations to MenC were measured for all subjects prior to vaccination. Post-vaccination rSBA titers and antibody concentrations were analyzed in 1192 and 1190 subjects, respectively. Reasons for exclusion included insufficient volume for testing and failure to pass rSBA acceptance criteria for a reportable titer. The number of serum samples excluded was similar between vaccine groups.

3.1. MenC serologic responses

Baseline rSBA GMT was <8 for both vaccine groups (Fig. 1a). Five to 7 months after vaccination, rSBA GMT increased to 703 and 1557 in MenACWYD and MPSV4 groups, respectively. GMT decreased by >93% for both groups by 3 years post-vaccination (GMT 43 and 85 for MenACWYD and MPSV4 groups, respectively). There were no significant differences in GMT between vaccine groups except at 5–7 months post-vaccination (adjusted P<0.05). GMC were <0.20 μg/mL for both vaccine groups prior to vaccination (Fig. 1b). Anti-MenC GMC increased to 1.07 μg/mL in the MenACWYD group.
Table 1
Proportion of subjects with a serum bactericidal titer ≥8 and ≥128 to meningococcal serogroup C and a 4-fold rise compared to baseline by time since vaccination with MenACWYD or MPSV4.

<table>
<thead>
<tr>
<th>Months after vaccination</th>
<th>Number of subjects</th>
<th>Percent with rSBA titer ≥8</th>
<th>Percent with rSBA titer ≥128</th>
<th>Percent with ≥4-fold rise compared to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MenACWYD</td>
<td>MPSV4</td>
<td>MenACWYD</td>
<td>MPSV4</td>
</tr>
<tr>
<td>Pre</td>
<td>600</td>
<td>600</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>5–7</td>
<td>99</td>
<td>99</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
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<td>73</td>
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<tr>
<td>35–37</td>
<td>100</td>
<td>100</td>
<td>54</td>
<td>62</td>
</tr>
</tbody>
</table>

and 6.00 µg/mL in the MPSV4 group 5–7 months after vaccination. By 3 years post-vaccination, GMC decreased by 38% (0.66 µg/mL) for MenACWYD and 51% (2.95 µg/mL) for MPSV4. GMC were significantly different between vaccine groups for all time-points, with the conjugate vaccine resulting in lower IgG antibody concentrations than the polysaccharide vaccine (adjusted P < 0.0035).

3.2. Proportion of subjects above a given threshold for Men C

The percentages of subjects achieving a serum bactericidal titer of ≥8 and ≥128 against MenC and a 4-fold rise compared to baseline are shown in Table 1. The proportion of subjects in both vaccine groups with titers ≥8 and ≥128 at 3 years compared to 5–7 months post-vaccination decreased by 29–38% and 35–43%, respectively. There were no significant differences between vaccine groups in the proportion of subjects with a titer of ≥8, ≥128, or 4-fold increase from baseline at any time-point. The proportion of subjects with MenC antibody concentrations ≥2.0 µg/mL was significantly lower in the MenACWYD group at all post-vaccination time-points (Table 2).

4. Discussion

This large-scale observational study of antibody persistence to MenC in U.S. military personnel demonstrates waning of immunity within 3 years following vaccination with either MenACWYD or MPSV4. No significant differences between the two vaccine groups were observed in the percentage of subjects at or above an rSBA titer of 8, the putative protective level for MenC. Lower antibody concentrations among MenACWYD recipients may be explained by the quantity of antigen in each vaccine as MenACWYD contains 1/10 the serogroup C antigen of MPSV4. Despite lower antibody concentrations, functional activity (as measured by rSBA) was comparable between vaccine groups, suggesting other immunologic responses elicited by conjugate vaccines likely contribute to overall protection. Conjugate vaccines are T-dependent and therefore induce immunologic memory and more rapid anamnestic responses after repeated doses, with antibody level typically several folds greater than after initial vaccination. This boost response has been demonstrated after a repeat dose of MenACWYCRM 5 years after primary vaccination [21]. Additionally, avidity maturation following initial vaccination elicits highly specific antibodies with greater bactericidal activity, and could thus explain the similarity in rSBA response despite significantly different antibody concentrations between vaccine groups.

The importance of maintaining circulating antibodies to prevent MenC disease has been reported in monovalent MenC conjugate vaccine post-licensure studies in the United Kingdom [22,23]. Previously vaccinated subjects that developed MenC disease demonstrated antibody levels and bactericidal activity comparable to unvaccinated patients, despite evidence of immunologic memory [22]. Anamnestic responses can take up to 5 days to develop and thus may not be sufficiently rapid enough to prevent disease [23].

Several U.S. studies have evaluated MenC antibody persistence greater than 1 year following vaccination with quadrivalent conjugate vaccines in adolescents [7,8,21]. These studies were conducted as extension studies within clinical trials, evaluated antibody persistence in <500 subjects per study, and provided duration of antibody response for only one time-point post-vaccination. Serologic responses varied between studies and could be attributed to differences in study population, type of protein conjugate used in the vaccines, and assay methods and reagents (e.g., complement source). Despite these differences, these studies demonstrated waning of immunity to MenC of 60–72%, 35–80%, and 56% at 2, 3 and 5 years after vaccination, respectively. Additionally, one of the studies suggested early evidence of waning at 2 years following vaccination MenACWYD and MenACWYCRM, regardless of brand type [7]. Evaluation of immune responses during the first month following vaccination was not conducted in our study; however, we were able to demonstrate an antibody decay of over 93% in anti-MenC serum bactericidal activity and over 38% in antibody concentrations during multiple sampling periods between 6 months and 3 years following vaccination with MenACWYD.

Preliminary estimates from a quadrivalent meningococcal conjugate vaccine effectiveness (VE) study conducted in over 60% of the US population demonstrates a decrease in VE from 82% (CI = 54%–93%) for adolescents vaccinated <1 year earlier to 59%

Table 2
Proportion of subjects with antibody concentration ≥2 µg/mL to meningococcal serogroup C by time since vaccination with MenACWYD or MPSV4.

<table>
<thead>
<tr>
<th>Months after vaccination</th>
<th>Number of subjects</th>
<th>Percent with anti-MenC IgG ≥2 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MenACWYD</td>
<td>MPSV4</td>
</tr>
<tr>
<td>Pre</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>5–7*</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>11–13</td>
<td>99</td>
<td>99</td>
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<tr>
<td>17–19</td>
<td>100</td>
<td>99</td>
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<td>23–25</td>
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<td>99</td>
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<tr>
<td>29–31</td>
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<td>98</td>
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<tr>
<td>35–37</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* P < 0.05, level of significance.
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Conflict of interest. The authors report no conflicts of interest.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

References


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Pre-vaccination rabbit serum bactericidal assay (rSBA) geometric mean titer (GMT) was <1:8 for the MenACWY<sub>D</sub> group. rSBA GMT increased to 703 at 5-7 months post-vaccination and decreased by 90% at 3 years post-vaccination. GMTs were significantly lower in the MenACWY<sub>D</sub> group at 11–13 and 17–19 months post-vaccination compared with the MPSV4 group. The percentage of MenACWY<sub>D</sub> recipients achieving a rSBA titer of ≥1:8 decreased from 87% at 5-7 months to 54% at 3 years. There were no significant differences in the proportion of subjects with a titer of ≥1:8 at any time point between vaccine groups. Geometric mean concentrations (GMCs) decreased from 1.1 µg/mL at 5-7 months to 0.6 µg/mL at 3 years in the MenACWY<sub>D</sub> group and were significantly lower than GMCs in the MPSV4 group at all time points.