

Antibody persistence and immune memory 15 months after priming with an investigational tetravalent meningococcal tetanus toxoid conjugate vaccine (MenACWY-TT) in toddlers and young children

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Abbreviations: ATP, according to protocol; CI, confidence interval; GMT, geometric mean antibody titres; MenACWY-TT, meningococcal tetravalent serogroups A,C,W-135 and Y vaccine with all serogroups conjugated to the tetanus toxoid (TT) carrier protein; MenC-CRM₁₉₇, meningococcal monovalent serogroup C vaccine conjugated to the mutant diphtheria toxoid (CRM₁₉₇) carrier protein; MenC-TT, meningococcal monovalent serogroup C vaccine conjugated to TT as carrier protein; MenPS, meningococcal tetravalent polysaccharide vaccine; rSBA, serum bactericidal antibody assays using baby rabbit serum as exogenous complement source; (S)AE, (serious) adverse event; SD, standard deviation

The present extension study, conducted in children originally vaccinated at 12–14 mo or 3–5 y of age, assessed antibody persistence and immune memory induced by an investigational tetravalent meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine (MenACWY-TT). In the original study, participants were randomized to receive one dose of MenACWY-TT or licensed age-appropriate meningococcal control vaccines. Fifteen months post-vaccination, all participants underwent serum sampling to evaluate antibody persistence and participants previously vaccinated as toddlers received a polysaccharide challenge to assess immune memory development.

Exploratory comparisons showed that (1) All children and $\geq 92.3\%$ of the toddlers maintained serum bactericidal (rSBA) titers $\geq 1:8$ at 15 mo post MenACWY-TT vaccination; statistically significantly higher rSBA geometric mean titers (GMTs) were observed compared with control vaccines. (2) At one month after polysaccharide challenge, all toddlers primed with MenACWY-TT or with the monovalent serogroup C conjugate vaccine had rSBA titers $\geq 1:8$ and $\geq 1:128$ for serogroup C and similar rSBA-GMTs; rSBA-GMTs for serogroups A, W-135 and Y were statistically significantly higher in toddlers primed with MenACWY-TT compared with the control vaccine. Thus, a single dose of MenACWY-TT induced persisting antibodies in toddlers and children and immune memory in toddlers.

This study has been registered at www.clinicaltrials.gov NCT00126984.

Infections caused by *Neisseria meningitidis* can be devastating, with case fatality rates of 10–15% and up to 20% of the survivors developing long-term sequelae.^{1,2} Meningococci are classified into 13 serogroups on the basis of the capsular polysaccharides; of these, six cause the majority of disease: MenA, MenB, MenC, MenW-135, MenY, and more recently, MenX.¹ Vaccination is the best strategy to prevent meningococcal diseases and meningococcal plain polysaccharide vaccines have been available for this purpose for many years. However, these vaccines may induce hyporesponsiveness, at least for some serogroups, do not elicit long-term protection or immune memory and are poorly

immunogenic in young children, who are at highest risk.²⁻⁴ Immunogenicity of the meningococcal vaccines can be increased or enabled by conjugation of the polysaccharides to carrier proteins, as first demonstrated by monovalent MenC conjugate vaccines.⁵ Currently, two tetravalent meningococcal conjugate vaccines offering protection against serogroups A, C, W-135 and Y, using diphtheria toxoid or a non-toxic cross-reacting mutant of diphtheria toxoid (CRM₁₉₇) as carrier proteins, have been licensed in various countries.

In addition, an investigational tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine, using tetanus

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toxoid (TT) as carrier protein (MenACWY-TT) has been shown to be immunogenic and to have a clinically acceptable safety profile in toddlers, children, adolescents, and young adults.⁶⁻¹² The present study evaluated the persistence of the immune response in toddlers and children 15 mo after priming with a single dose of MenACWY-TT. In addition, participants who were vaccinated as toddlers received a reduced dose of meningococcal polysaccharide vaccine to mimic exposure to meningococcal bacteria and to assess whether immune memory had been induced.

This phase II, open, controlled study conducted in 30 centers in Germany and five centers in Austria between November 2006 and February 2008 was an extension of the previously reported study evaluating four different formulations of MenACWY-TT.⁶ The extension study compared the antibody persistence and the immune memory induced by the MenACWY-TT formulation containing 5 µg of each capsular polysaccharide conjugated to TT (-44 µg) to that of licensed age-appropriate control vaccines. The randomization ratio was 1:1 for these two groups in the primary study.⁶ The control vaccine was a monovalent MenC conjugate vaccine using mutant diphtheria toxoid (CRM₁₉₇) as carrier protein (*Meningitec*[™], Pfizer, hereafter referred as MenC-CRM₁₉₇) for the toddlers aged 12–14 mo at the time of vaccination or a tetravalent meningococcal serogroups A, C, W-135 and Y plain polysaccharide vaccine (*Mencevax*[™] ACWY, GlaxoSmithKline Biologicals, hereafter referred as MenPS) for the children aged 3–5 y at the time of vaccination.

Participants from the primary study were not included in the extension study if they had received a meningococcal vaccine not planned in the protocol, immunoglobulin, blood products, any investigational product, or immune-modifying drug during the study period. Written informed consent was obtained from each parent/guardian prior to study entry. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and the protocol and informed consent were approved by national or regional ethics committees. This study has been registered at www.clinicaltrials.gov NCT00126984.

Blood samples were collected from all the participants at 15 mo post-primary vaccination. Participants who were vaccinated as toddlers in the primary study received a polysaccharide challenge (1/5 dose of MenPS, or a 10 µg dose of the capsular polysaccharides for meningococcal serogroups A, C, W-135 and Y) and an additional blood sample was collected from these participants one month later. The choice of 1/5th dose of MenPS was selected based on the design of a previous study, in which 1/5 dose of a bivalent polysaccharide vaccine against meningococcal serogroups A and C (*Mengivac A + C*[®]; Sanofi Pasteur Mérieux) containing 10 µg of each meningococcal serogroup was administered in children to demonstrate that monovalent meningococcal serogroup C conjugate vaccines induced immune memory.¹³ Immune memory was not assessed in the cohort vaccinated at 3–5 y of age because of concerns of inducing hyporesponsiveness after a second vaccination with meningococcal polysaccharide vaccines in the control group.⁴

Immunogenicity analyses were conducted on the according to protocol (ATP) cohort for persistence at 15 mo post-primary

vaccination and on the ATP cohort for immune memory at one month after polysaccharide challenge administration. Functional antibody responses against the four serogroups were assessed by serum bactericidal antibody assays using baby rabbit serum as complement source (rSBA).¹⁴ The cut-off of the assay was a rSBA titer $\geq 1:8$, which has been shown to correlate with protection for serogroup C, and evaluation of this threshold was extended to the other serogroups.^{15,16} The percentages of participants with rSBA titers $\geq 1:128$, which is the more conservative threshold to define seroprotection, were also evaluated.^{17,18} An exploratory statistical analysis was performed to evaluate the potential differences between the MenACWY-TT and the control groups. Exploratory analyses were supplementary analyses that were not prospectively stated as primary objectives of the study, but were nonetheless pre-defined in the study protocol and statistical analysis plan. Two groups were considered statistically significantly different if the asymptotic standardized 95% confidence interval (CI) for (1) the difference in percentage of participants with titers above the proposed cut-offs between the two groups did not contain the value “0” or (2) the GMT ratio adjusted for pre-vaccination titers between the two groups did not contain the value “1.”. Statistically significant findings must be interpreted with caution given their exploratory nature. As the study was not powered to evaluate the exploratory objectives and there was no adjustment for multiplicity of endpoints, the relevance of potential statistically significant differences is not clear.

Local and general solicited adverse events (AEs) were recorded for seven days and unsolicited AEs for 30 d following administration of the polysaccharide challenge by the parent/guardian on a standardised diary card. Occurrence of any serious adverse event (SAE) was recorded throughout the entire study. The statistical analyses were performed using the SAS[®] software version 9.1 (SAS Institute Inc.) and StatXact 7.0 (Cytel).

At 15 mo post-primary vaccination, 185 of the 203 eligible participants were enrolled in the extension study (Fig. 1). Demographic characteristics of both groups were comparable within each age stratum and consistent with the primary study (data not shown).⁶

All the children vaccinated with MenACWY-TT and 59.4% to 93.8% of those who received MenPS at 3–5 y of age in the primary study had persisting rSBA titers $\geq 1:8$ for the individual serogroups at 15 mo post-primary vaccination (Table 1). Between one month and 15 mo post-vaccination, the rSBA GMTs for MenA, MenC, MenW-135 and MenY decreased 4.7-, 7.8-, 6.1-, and 5.2-fold, respectively, in the children vaccinated with MenACWY-TT and 7.0-, 15.9-, 7.5- and 10.0-fold, respectively, in the children vaccinated with MenPS (Fig. 2). Exploratory analyses showed statistically significantly higher rSBA GMTs for all serogroups and percentages of children with rSBA titers $\geq 1:8$ and $\geq 1:128$ for MenA, MenC and MenY in the MenACWY-TT recipients compared with the MenPS recipients (Table S2). These results are in line with those of the primary study, which showed that rSBA GMTs against each serogroup were statistically higher at one month post-MenACWY-TT vaccination compared with MenPS.⁶ These findings were expected as it has previously been observed that conjugate vaccines induce a more robust immune

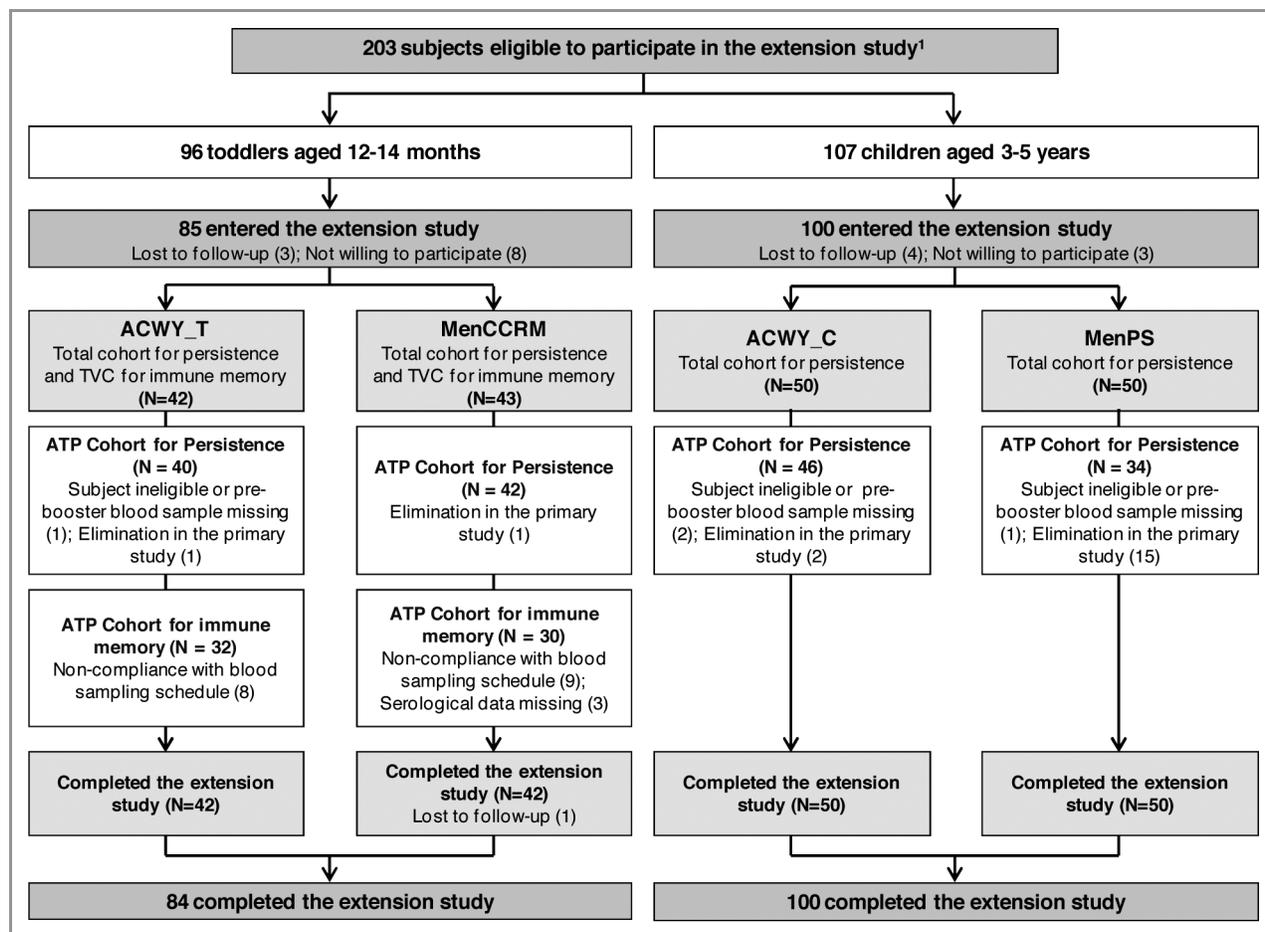


Figure 1. Participant flow. ACWY_T, toddlers vaccinated with MenACWY-TT at 12–14 mo of age; MenCCRM, toddlers vaccinated with MenC-CRM₁₉₇ at 12–14 mo of age; ACWY_C, children vaccinated with MenACWY-TT at 3–5 y of age; MenPS, children vaccinated with MenPS at 3–5 y of age; ATP, according to protocol; TVC, total vaccinated cohort; N, number of participants. ¹Several formulations were evaluated in the primary study, but participants who received the selected formulation for further development or control vaccine were eligible to participate in the persistence study. Participants who withdrew from the primary phase were described in the previous publication.⁶

response than polysaccharide vaccines with a slower rate of decay of GMTs.^{2,3,19}

The percentages of toddlers vaccinated at 12–14 mo of age who had persisting rSBA-MenC titers $\geq 1:8$ at 15 mo post-primary vaccination were 92.3% and 60.0% in the MenACWY-TT and MenC-CRM₁₉₇ recipients, respectively (Table 1). The decrease in rSBA-MenC GMTs between 1 and 15 mo post-vaccination was less for in the toddlers who received the MenACWY-TT vaccine than with the MenC-CRM₁₉₇ vaccine (5.4-fold vs. 15-fold) (Fig. 2). Exploratory analyses for the common antigen between the two groups showed statistically significantly higher proportions of toddlers with rSBA-MenC titers $\geq 1:8$ or $\geq 1:128$ and rSBA-MenC GMTs at 15 mo after vaccination with MenACWY-TT compared with MenC-CRM₁₉₇ (Table S2). This observation is consistent with results of a previous study in which a monovalent vaccine using TT as carrier (MenC-TT) was compared with two monovalent vaccines using CRM₁₉₇ as carrier protein.^{13–20} In the toddlers vaccinated with MenACWY-TT, the rSBA GMTs for MenA, MenW-135 and MenY decreased 4.3-, 8.2- and 4.4-fold, respectively, between one month and 15 mo

post-vaccination (Fig. 2). At 15 mo post-primary vaccination, rSBA GMTs for MenA, MenW-135 and MenY induced by MenACWY-TT were statistically significantly higher than antibodies derived from natural immunity, as measured in toddlers primed with MenC-CRM₁₉₇ (Table S2).

All toddlers who were primed with MenACWY-TT or MenC-CRM₁₉₇ in the primary study, had rSBA-MenC titers $\geq 1:8$ and $\geq 1:128$ at one month post-challenge in the extension study (Table 1). Similar rSBA-MenC GMTs were observed in both groups after the administration of the polysaccharide challenge, which induced a 34.3- and 153.1-fold increase in rSBA-MenC GMTs in the MenACWY-TT vaccine and the MenC-CRM₁₉₇ control vaccine, respectively (Fig. 2; Table S2). This difference in fold-increases for rSBA-MenC GMTs was driven by the lower persistence in the group originally vaccinated with MenC-CRM₁₉₇. Thus, both the MenACWY-TT vaccine and the MenC-CRM₁₉₇ control vaccine were observed to prime for immunological memory. These results confirm those of a previous study, in which three MenC conjugate vaccines induced immunologic memory after a single dose in toddlers.¹³

Table 1. Percentage of participants with rSBA titers above defined thresholds at pre-vaccination, at 1 mo and 15 mo after primary vaccination (ATP cohort for persistence) and at 1 mo after the polysaccharide challenge (ATP cohort for immune memory)

		Toddlers			Children		
		N	% ≥ 1:8 [95% CI]	% ≥ 1:128 [95% CI]	N	% ≥ 1:8 [95% CI]	% ≥ 1:128 [95% CI]
rSBA-MenA							
ACWY-TT	M0 ¹	37	10.8 [3.0, 25.4]	10.8 [3.0, 25.4]	39	20.5 [9.3, 36.5]	15.4 [5.9, 30.5]
	M1 ¹	38	100 [90.7, 100]	100 [90.7, 100]	46	100 [92.3, 100]	100 [92.3, 100]
	M15	40	97.5* [86.8, 99.9]	92.5* [79.6, 98.4]	45	100* [92.1, 100]	97.8* [88.2, 99.9]
	M16	25	100 [86.3, 100]	100 [86.3, 100]	-	-	-
Control	M0 ¹	39	2.6 [0.1, 13.5]	0.0 [0.0, 9.0]	27	33.3 [16.5, 54.0]	33.3 [16.5, 54.0]
	M1 ¹	41	2.4 [0.1, 12.9]	2.4 [0.1, 12.9]	33	100 [89.4, 100]	100 [89.4, 100]
	M15	36	30.6 [16.3, 48.1]	27.8 [14.2, 45.2]	29	89.7 [72.6, 97.8]	82.8 [64.2, 94.2]
	M16	30	100 [88.4, 100]	90.0 [73.5, 97.9]	-	-	-
rSBA-MenC							
ACWY-TT	M0 ¹	39	10.3 [2.9, 24.2]	2.6 [0.1, 13.5]	44	22.7 [11.5, 37.8]	18.2 [8.2, 32.7]
	M1 ¹	38	100 [90.7, 100]	97.4 [86.2, 99.9]	46	100 [92.3, 100]	95.7 [85.2, 99.5]
	M15	39	92.3* [79.1, 98.4]	69.2* [52.4, 83.0]	46	100* [92.3, 100]	65.2* [49.8, 78.6]
	M16	32	100 [89.1, 100]	100 [89.1, 100]	-	-	-
Control	M0 ¹	40	10.0 [2.8, 23.7]	2.5 [0.1, 13.2]	32	37.5 [21.1, 56.3]	18.8 [7.2, 36.4]
	M1 ¹	42	97.6 [87.4, 99.9]	88.1 [74.4, 96.0]	34	97.1 [84.7, 99.9]	88.2 [72.5, 96.7]
	M15	40	60.0 [43.3, 75.1]	27.5 [14.6, 43.9]	32	59.4 [40.6, 76.3]	28.1 [13.7, 46.7]
	M16	30	100 [88.4, 100]	100 [88.4, 100]	-	-	-
rSBA-MenW-135							
ACWY-TT	M0 ¹	28	39.3 [21.5, 59.4]	25.0 [10.7, 44.9]	31	54.8 [36.0, 72.7]	35.5 [19.2, 54.6]
	M1 ¹	28	100 [87.7, 100]	100 [87.7, 100]	31	100 [88.8, 100]	100 [88.8, 100]
	M15	40	97.5* [86.8, 99.9]	90.0* [76.3, 97.2]	46	100 [92.3, 100]	100 [92.3, 100]
	M16	32	100 [89.1, 100]	100 [89.1, 100]	-	-	-
Control	M0 ¹	31	29.0 [14.2, 48.0]	19.4 [7.5, 37.5]	19	57.9 [33.5, 79.7]	31.6 [12.6, 56.6]
	M1 ¹	31	32.3 [16.7, 51.4]	22.6 [9.6, 41.1]	19	100 [82.4, 100]	100 [82.4, 100]
	M15	41	41.5 [26.3, 57.9]	26.8 [14.2, 42.9]	32	93.8 [79.2, 99.2]	93.8 [79.2, 99.2]
	M16	30	100 [88.4, 100]	96.7 [82.8, 99.9]	-	-	-

Table 1. Percentage of participants with rSBA titers above defined thresholds at pre-vaccination, at 1 mo and 15 mo after primary vaccination (ATP cohort for persistence) and at 1 mo after the polysaccharide challenge (ATP cohort for immune memory) (continued)

		Toddlers			Children		
		rSBA-MenY					
ACWY-TT	M0 ¹	38	52.6 [35.8, 69.0]	28.9 [15.4, 45.9]	45	75.6 [60.5, 87.1]	57.8 [42.2, 72.3]
	M1 ¹	38	100 [90.7, 100]	100 [90.7, 100]	46	100 [92.3, 100]	100 [92.3, 100]
	M15	40	97.5* [86.8, 99.9]	90.0* [76.3, 97.2]	46	100* [92.3, 100]	100* [92.3, 100]
	M16	32	100 [89.1, 100]	100 [89.1, 100]	-	-	-
Control	M0 ¹	40	42.5 [27.0, 59.1]	17.5 [7.3, 32.8]	33	60.6 [42.1, 77.1]	45.5 [28.1, 63.6]
	M1 ¹	41	46.3 [30.7, 62.6]	31.7 [18.1, 48.1]	34	100 [89.7, 100]	97.1 [84.7, 99.9]
	M15	40	75.0 [58.8, 87.3]	45.0 [29.3, 61.5]	33	78.8 [61.1, 91.0]	66.7 [48.2, 82.0]
	M16	30	96.7 [82.8, 99.9]	93.3 [77.9, 99.2]	-	-	-

ATP, according to protocol; Toddlers, participants 12–14 mo of age at the time of the primary vaccination; Children, participants 3–5 y of age at the time of the primary vaccination; ACWY-TT, participants primed with MenACWY-TT; control, participants primed with MenC-CRM₁₉₇ in the toddlers age stratum or with MenPS in the children age stratum; N, number of participants with available results; %, percentage of participants with titer within the specified range; 95% CI, exact 95% confidence interval; M0, pre-primary vaccination; M1, one month post-primary vaccination; M15, 15 mo post-primary vaccination; M16, one month post-polysaccharide challenge. ¹These numbers are not the same as those presented in the previous publication at the same timepoints because the analyses for the present study were conducted on the ATP cohort for persistence (Month 15) and in the previous publication the analyses were performed on the ATP cohort for immunogenicity (Month 1). *Statistically significantly higher values compared with the control group (exploratory analysis).

All the toddlers vaccinated with MenACWY-TT in the primary study had rSBA titers $\geq 1:128$ for MenA, MenW-135 and MenY at one month after administration of the polysaccharide challenge and this percentage ranged between 90.0% and 96.7% in the toddlers who received MenC-CRM₁₉₇ in the primary study (Table 1). In the toddlers who were primed with MenACWY-TT, the polysaccharide challenge induced a 6.1-, 17.2- and 13.0-fold increase in rSBA GMTs for MenA, MenW-135, and MenY, respectively (Fig. 2). Exploratory analyses showed that post-polysaccharide challenge GMTs for MenA, MenW-135 and MenY were statistically significantly higher in the toddlers primed with MenACWY-TT compared with those who received MenC-CRM₁₉₇ in the primary study and were unprimed for these serogroups (Table S2), suggesting the induction of immune memory in the recipients of MenACWY-TT for these serogroups.

The incidence of local reactions after administration of the polysaccharide challenge was similar in toddlers primed with MenACWY-TT or MenC-CRM₁₉₇ (data not shown). One grade 3 solicited local symptom and three grade 3 solicited general symptoms were reported in toddlers primed with MenC-CRM₁₉₇. Four participants experienced one or more SAEs throughout the study, but none were considered related to vaccination.

A potential limitation of this study was its open design, which would not have influenced the immunogenicity results because the laboratory personnel was blinded during immunological assays but may have induced a bias in the safety profile of the

polysaccharide challenge. Theoretically, functional antibody levels could have been biased if participants were exposed to *N. meningitidis* during the 15-mo post-vaccination follow-up. Although there is limited epidemiological information on the seroprevalence of antibodies against meningococcal serogroups A, C, W-135 and Y in Europe, some results are available for the United Kingdom (UK). Before the introduction of monovalent meningococcal serogroup C conjugate vaccines, it was observed that the majority of individuals lacked functional antibody titers against this serogroup and the lowest titers were found in young children.²¹ After the introduction of the meningococcal serogroup C conjugate vaccines, the prevalence of protective antibodies against meningococcal serogroup C increased from 10–15% between 1996 and 1999 to 32% between 2000 and 2004 among children between one and five years of age.^{21,22} A more recent study conducted in 2009 showed that natural immunity against meningococcal serogroups W-135 and Y was detected in 7% and 13% of children younger than five years of age, respectively.²³

Some results are also available for Turkey, where a previous study showed that, regardless of age, 60.5%, 27.2%, 12.3% and 19.2% of inhabitants had serogroup-specific antibody concentrations $\geq 2 \mu\text{g/mL}$ for serogroups A, C, W-135 and Y, respectively,²⁴ but these data may not correlate with functional bactericidal activity. Christensen, et al., performed a meta-analysis of meningococcal carriage, and concluded that rates of meningococcal carriage are lowest in infants (4.5%) and increase to 7.7% in children 10 y of age.²⁵ Given the limited evidence for extensive carriage and seroprevalence of functional anti-meningococcal

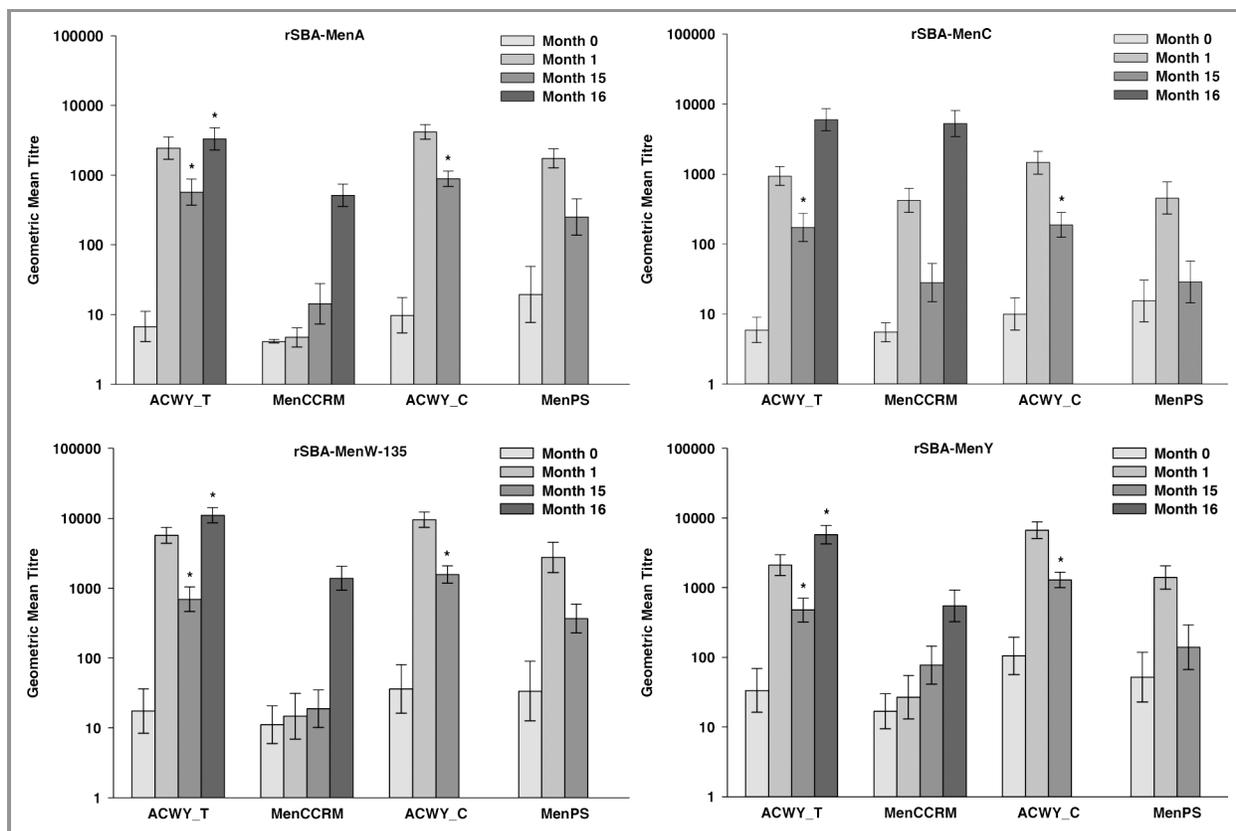


Figure 2. Geometric mean titers for rSBA at pre-vaccination, at 1 mo¹ and 15 mo¹ after primary vaccination (ATP cohort for persistence) and at 1 mo after the polysaccharide challenge (ATP cohort for immune memory). Notes: ATP, according to protocol; ACWY_T, toddlers primed with MenACWY-TT at 12–14 mo of age; MenCCRM, toddlers primed with MenC-CRM₁₉₇ at 12–14 mo of age; ACWY_C, children primed with MenACWY-TT at 3–5 y of age; MenPS, children primed with MenPS at 3–5 y of age; errors bars, exact 95% confidence interval; Month 0, pre-primary vaccination; Month 1, 1 mo post-primary vaccination; Month 15, 15 mo post-primary vaccination; Month 16, one month post-polysaccharide challenge. ¹These numbers are not the same as those presented in the previous publication at the same timepoints because the analyses for the present study were conducted on the ATP cohorts for persistence and immune memory (Month 15) and in the previous publication the analyses were performed on the ATP cohort for immunogenicity (Month 1). *Statistically significantly higher values compared with the control group (exploratory analysis).

antibodies in young children, it is likely that boosting from circulating meningococci had a limited impact on the antibody persistence observed in this study.

In conclusion, this extension study showed the persistence of the antibody responses (rSBA \geq 1:8) induced by MenACWY-TT for 15 mo after vaccination in > 92% of the toddlers aged 12–14 mo and in all the children aged 3–5 y. Moreover, this study showed that a single dose of MenACWY-TT was able to induce immune memory for the four serogroups included in the vaccine. This observation confirms and extends the results of a previous study showing that a single dose of three different monovalent meningococcal serogroup C vaccines in toddlers induced the development of immune memory.¹³

Meningitec is a trademark of Pfizer, formerly Wyeth; Mencevax is a trademark of the GlaxoSmithKline Biologicals group of companies.

Disclosure of Potential Conflicts of Interest

M.K. received honoraria or consulting fees as well as support for meetings, travel or accommodation expenses from GSK in the

past three years. Y.B., V.B., D.B. and J.M. are employees of GSK Biologicals. Y.B., D.B. and J.M. declare stock ownership in GSK. D.B. is also inventor of certain GSK Biologicals patents.

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Supplemental Material

Supplemental materials may be found here:
www.landesbioscience.com/journals/vaccines/article/20229

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