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The B-cell response to a primary and booster course of MenACWY-CRM$_{197}$ vaccine administered at 2, 4 and 12 months of age

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ABSTRACT

A quadrivalent meningococcal vaccine conjugated to CRM$_{197}$ (MenACWY-CRM$_{197}$) is immunogenic in young infants. We assessed the memory B-cell and antibody responses after a primary and booster course of MenACWY-CRM$_{197}$ in children. At 5 months of age, following primary immunisation, serogroup-specific memory B-cells were detectable in fewer than 25% of children, although protective antibody titres (hSBA $\geq$ 4) were detectable in 69% of children against serogroup A and more than 95% against the other serogroups. At 12 months, before booster immunisation the percentages with hSBA $\geq$ 4 were 5% for serogroup A, and between 44 and 70% for the other serogroups. One month after booster immunisation with MenACWY-CRM$_{197}$ over 50% of children had detectable memory B-cells, and 91% had hSBA $\geq$ 4 against serogroup A and more than 99% against the other serogroups. These data show that few antigen-specific anticapsular memory B-cells can be detected after two-doses priming with MenACWY-CRM$_{197}$. For MenC and CRM$_{197}$, the antigens with the highest number of B-cells at 5 months, there was a definite ($p \leq 0.02$) but weak correlation with antibody persistence at 12 months. Although previous studies suggest that measuring memory B-cell responses after priming immunisations in infancy can be used to predict antibody persistence and memory responses, this may not be suitable for all antigens in young children.

1. Introduction

A tetravalent serogroups A, C, W and Y meningococcal protein polysaccharide vaccine conjugated to CRM$_{197}$ (MenACWY-CRM$_{197}$) is licensed in the US in persons 2–55 years of age and EU for use in persons above 2 years of age. The MenACWY-CRM$_{197}$ vaccine is immunogenic in young infants, inducing antibody responses against the four serogroups in more than 80% of young children [1,2]. Similar to serogroup C meningococcal glycoconjugate vaccine (MenCV), following MenACWY-CRM$_{197}$ priming in early infancy, a decline in protective antibodies was observed less than one year after immunisation despite the induction of immunologic memory as measured by the booster response [2]. Long-term protection against invasive meningococcal disease relies on the persistence of bactericidal antibodies in serum [3,4]. Studies of B-cell responses to immunisation in animal models suggest that the generation of memory B-cells and long-lived plasma cells during primary immunisation may be the principal determinant of long-term maintenance of antibody, as these cells have been shown to persist and to contribute to antibody secretion [5,6]. However, in humans the organs of formation and residence of these cells are not easily accessible, and B-cells can only be readily assessed in peripheral blood [7]. In this study, memory B-cell and antibody responses specific to the four serogroups of meningococci were determined one month after a two-dose primary course of MenACWY-CRM$_{197}$ vaccine, and before and after a booster dose at 12 months of age. The primary objective of this study was

Abbreviations: CRM$_{197}$, cross reactive material (CRM$_{197}$: mutant peptide related to diphtheria toxoid); DTaP-Hib-IPV, combined diphtheria toxoid, tetanus toxoid, acellular pertussis, Haemophilus influenzae type b, and inactivated polio vaccine; GMC, geometric mean concentration; GMT, geometric mean titre; MenACWY-CRM$_{197}$, tetravalent serogroups A, C, W and Y meningococcal protein polysaccharide vaccine conjugated to CRM$_{197}$; PCV, pneumococcal conjugate vaccine (PCV-7: 7-valent pneumococcal conjugate vaccine); SBA, serum bactericidal activity (hSBA with human complement).

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to assess whether the frequency of polysaccharide-specific memory B-cells, measured at 5 months of age, after priming with MenACWY-CRM_{197} vaccine, predicts the hSBA titers against the four serogroups of meningococci at 12 months of age. A correlation between memory B-cells at 5 months and antibody persistence at 12 months would confirm the major role of memory B-cells in driving long-term antibody persistence of immunity. If this relationship is reproducible, the assessment of memory B-cell responses in addition to antibody responses should be used in evaluation of vaccines to provide a better indication of long-term protection.

2. Materials and methods

2.1. Study population

A phase II, single centre, open-label, randomised study was conducted in Oxford, UK, between May 2007 and December 2009, to assess the serogroup-specific B-cell responses to a primary and booster course of MenACWY-CRM_{197} vaccine (Menveo, Novartis Vaccines and Diagnostics Srl, Siena). Potential participants were recruited by information letter in the Thames Valley region, UK. Exclusion criteria included previous meningococcal disease or household contact with meningococcal disease, immune dysfunction, recent receipt of antibiotics or corticosteroids. Written informed consent was obtained from the mothers of all enrolled infants. Ethical approval was obtained from the Oxfordshire Research Ethics Committees (approval number B07/Q1605/41; Eudract number 2006-003476-35). The trial was registered with clinicaltrials.gov (identifier NCT 00488683). Participating infants were randomised to one of three groups in a 2:1:1 ratio to allow assessment of immunogenicity at various time-points whilst minimizing the number of blood samples obtained from each child.

2.2. Immunisation procedures

One dose of 0.5 ml of the MenACWY-CRM_{197} vaccine was administered by intramuscular injection into the right anterolateral thigh at 2, 4 and 12 months of age. The 0.5 ml dose consisted of *N. meningitidis* serogroup A, C, Y and W capsular saccharides (10 μg of serogroup A; 5 μg each of the other serogroups) individually conjugated to CRM_{197}, without adjuvant. At the same time, as per the routine UK immunisation schedule, concomitant vaccines were administered into the left thigh; the combined diphtheria toxoid, tetanus toxoid, acellular pertussis, *Haemophilus influenzae* type b (Hib), and inactivated polio vaccine (Pediacel, Aventis-Pasteur MSD Ltd) at 2, 3 and 4 months of age, and the 7-valent pneumococcal conjugate vaccine (PCV-7, Prevenar, Wyeth Vaccines) at 2 and 4 months of age (Table 1).

In addition group I received the PCV-7 at 13 months of age and groups II and III received the PCV-7 concomitantly with the MenACWY-CRM_{197} at 12 months of age. Furthermore the combined measles, mumps and rubella vaccine (MMR) and a booster dose of Hib were offered on the completion of the study at 13 months of age according to the routine UK immunisation schedule at the time (Table 1).

2.3. Sampling procedures

For all children, blood samples were taken at 5 months, following priming with MenACWY-CRM_{197} at 2 and 4 months of age (visit 4), immediately before the third immunisation with MenACWY-CRM_{197} at 12 months (visit 5), 1 month following the 12-months immunisation (visit 6). In addition, participants had one additional blood draw at a time which was determined by their group: group I children were divided into 6 subgroups for the kinetics study, which involved a blood draw either before or at various days after the second dose of MenACWY-CRM_{197} at 4 months; group II had an additional blood draw at the time of enrolment (2 months); group III had an additional blood draw 6–7 days following the 12-months dose of MenACWY-CRM_{197} (Table 1).

2.4. Preparation of PBMCs

Up to 5 ml of heparinised blood was diluted 1:2 with RPMI-1640 medium (Sigma–Aldrich, England) to which penicillin-streptomycin solution (Sigma–Aldrich, England) and L-glutamine 200 mM (Sigma–Aldrich, England) had been added at a dilution of 1:100 (complete medium). The peripheral blood mononuclear cells (PBMCs) were then separated by density gradient centrifugation over Lymphoprep (Axis-Shield, Diagnostics, England). PBMCs were washed once in complete medium prior to further preparation for ELISpot or cell culture.

2.5. Preparation of ELISpot plates

ELISpot plates (96 well PVDF membrane) (Millipore, England) were coated with either serogroups A, C, W-135 and Y meningococcal polysaccharide (5 μg/ml) (NIBSC, UK) conjugated to methylated human albumin (5 μg/ml) (NIBSC, UK), or 10 μg/ml CRM_{197} (Novartis Vaccines, Siena, Italy), or 10 μg/ml goat anti-human Ig (Caltag laboratories, Burlingame, USA) in sterile phosphate buffered saline (PBS). PBS alone was added to the antigen blank wells. The ELISpot plates were stored at 4 degrees centigrade until use.

2.6. Detection of memory B-cells

PBMCs prepared from peripheral blood were re-suspended in complete medium with 10% foetal calf serum at a final concentration of 2×10^6 PBMCs/ml and 100 μl was added per well of a 96-well round bottomed culture plate (Greiner One-Bio Ltd, England). The cells were stimulated with medium, containing 1/5000 of *Staphylococcus aureus* Cowan strain suspension (SAC), (Calbiochem, England), 83 ng/ml Pokeweed mitogen (PWM) (Sigma–Aldrich, England) and 2.5 μg/ml CpG oligonucleotide (ODN-2006) (Source Bioscience, England). The cells were incubated at 37 °C in 5% CO_2 for 5.5 days before being re-suspended and washed 4 times in PBS-EDTA with 0.5% new born bovine serum and resuspended in complete medium with 10% foetal calf serum. The cultured cells were plated onto pre-coated ELISpot plates that had been previously blocked with complete medium with 10% foetal calf serum at 2×10^5 cells/well. The ELISpot plates containing the cells were then incubated overnight at 37 °C in 5% CO_2 and 95% humidity. The wells were washed with PBS-Tween and bound IgG antibodies detected using a 1:5000 dilution of goat anti-human IgG γ-chain specific alkaline phosphatase conjugate (Calbiochem, UK) in complete medium with 10% foetal calf serum for 4 h. Bound alkaline phosphatase conjugate was detected using the substrate kit (5-bromo-4-chloro-3-indolyl phosphate in nitroblue tetrazolium dissolved in aqueous dimethylformamide, Bio-Rad Laboratories, England). The reaction was stopped with sterile distilled water, 200 μl/well.

2.7. ELISpot counting

Spots were counted using an AID ELISpot Reader ELR02 (AID) and ELISpot software, version 3.2.3 (Cadama Medical Ltd, Stourbridge, UK). Spot-forming cells were counted and confirmed by visual inspection. Identical settings were used for all plates but different settings were used for the different antigens (polysaccharide antigens versus protein antigens). The operator was blinded to which sample was being counted.

Table 1
Study groups and number of subjects for each group.

Visit	1	2	3	4 (All Groups) 4 (Kinetics) (Group 1 only)	5 (Group 1 and 2) 5a (Group 3)	5b (Group 3 only)	6
Age/Group (no. subjects)	2 months	3 months	4 months	5 months (Group 1 variable)	12 months	6/7 days post V5a	13 months
1 (108)	Maternal venepuncture *MenACWY* DTaP-Hib-IPV PCV	DTaP-Hib-IPV	*MenACWY* DTaP-Hib-IPV PCV	Venepuncture (day 30 post V3)	Venepuncture *MenACWY*		Venepuncture Hib* MMR* PCV
				Venepuncture (kinetics) (visit timing according to subgroup allocation)			
2 (54)	Maternal venepuncture Venepuncture *MenACWY* DTaP-Hib-IPV PCV	DTaP-Hib-IPV	*MenACWY* DTaP-Hib-IPV PCV	Venepuncture (day 30 post V3)	Venepuncture *MenACWY* PCV		Venepuncture Hib* MMR*
3 (54)	Maternal venepuncture *MenACWY* DTaP-Hib-IPV PCV	DTaP-Hib-IPV	*MenACWY* DTaP-Hib-IPV PCV	Venepuncture (day 30 post V3)	Venepuncture *MenACWY* PCV	Venepuncture	Venepuncture Hib* MMR*

MenACWY = study vaccine MenACWY-CRM$_{197}$, DTaP-Hib-IPV = combined diphtheria toxoid, tetanus toxoid, acellular pertussis, Hib and inactivated polio vaccine Pediacel, PCV = 7-valent pneumococcal conjugate vaccine Prevenar

2.8. Anti-N.meningitidis serogroups A, C, W and Y-specific bactericidal activity as measured by hSBA

Serum bactericidal antibody (SBA) assays using human complement (hSBA) for meningococcal serogroups A, C, Y and W were performed at the laboratories of Novartis Vaccines, Marburg, Germany according to standard protocols [8,9]. Briefly, twofold dilutions of heat-inactivated sera were incubated with suspensions of meningococcal strains A, C, W and Y and freshly thawed human complement. The last dilution producing a ≥50% reduction in colonies (killing) compared to control wells, containing complement and bacteria, was taken as the end point hSBA titre. The reference strains used for the relevant serogroups were serogroup A, F8238; C, C11; W, M01-240070; and Y, 860800.

2.9. Anti-N. meningitidis serogroups A, C, Y and W antibody concentration as measured by ELISA

The concentration of IgG specific for meningococcal serogroups A, C, W and Y was determined by ELISA following a previously described method [10]. Briefly, immulon-2 microtiter plates (Thermo Electron Corporation, England) were coated with either serogroup A, C, W and Y meningococcal polysaccharide (5 μg/ml) (NIBSC, England) conjugated to 5 μg/ml methylated human albumin (NIBSC, England) in sterile PBS. Following blocking, eight twofold dilutions of the reference serum (CDC 1992, NIBSC 99/706); starting dilution 1:400 for serogroup A and 1:150 for serogroups C, W and Y) and test sera (starting dilution 1:25) were made directly in the microtiter plate by well-to-well transfer with a multichannel pipette. The reference serum was assayed in triplicate, and test sera were assayed in duplicate. Additionally an internal quality control (an anti-meningococcal adult immune serum) was diluted to yield optical densities approximately on the high, middle, and low portions of the reference curve. After overnight incubation at 4 °C, microtiter plates were developed with monoclonal-PAN anti-human Fc γ peroxidase antibody (diluted in serum/conjugate [S/C] buffer) (Stratech Scientific Ltd., England) for 2.5 h at room temperature, followed by the chromogenic substrate tetramethylbenzidine dihydrochloride monohydrate (Sigma–Aldrich, England), and the reaction was stopped after 30 min with 2 M H_2SO_4. The optical density of each well was then read at 450 nm. A sigmoid logistic plot of reference sera dilutions was used to calculate test sera antibody concentrations. The antibody concentrations were calculated in ng/ml from the sigmoid logistic plot using Revelation softwear (ThermalLabs Inc., Basingstoke, UK). All the data were then subsequently converted to mcg/ml for analysis.

2.10. Anti-CRM$_{197}$ IgG concentration

The CRM$_{197}$ IgG concentration was determined using an internally validated ELISA. In brief, Nunc Immuno Maxisorp microtitre plates (Thermo Fisher Scientific) were coated with 5.0 μg/ml of diphtheria toxin mutant CRM197 (Novartis, Siena). Following blocking and washing steps, serial dilutions of sample sera and a reference diphtheria antitoxin serum NIBSC (00/496), were made in duplicate. Following two hours of incubation, plates were washed and goat anti-human IgG-Fc specific alkaline phosphatase conjugate antibodies (Sigma–Aldrich) were added for one hour. Plates were once more washed and p-nitrophenyl phosphate (Sigma–Aldrich) added, after 20 min the enzymatic reaction was terminated with 3 M NaOH. The optical density of each well was read at 405 nm and results were reported in International Units (IU) per ml.

2.11. Statistical analysis

Intention to treat analysis was performed. For the purposes of analysis, ELISpot assays in which fewer than 4 spots were detected were treated as though no ASCs were detected. Normally, eight replicate wells were used for each meningococcal serogroup and CRM$_{197}$, and so the maximal sensitivity of the assay was 2.5 cells per million PBMCs. However, when the total number of PBMCs available was limited, at least 8 replicate wells were used for serogroup C and 4 replicate wells for CRM$_{197}$, and the rest of the cells were used to assess the other serogroups. A memory B cell frequency of less than 2.5 cells/million cultured lymphocytes was assigned the value "0". hSBA titre and ELISA IgG concentrations were summarised using geometric means. B-cell numbers were summarised by medians. The percentage of participants with hSBA titre ≥4 were calculated. Stata (version 9.1, StataCorp, USA) was used for the statistical analysis.

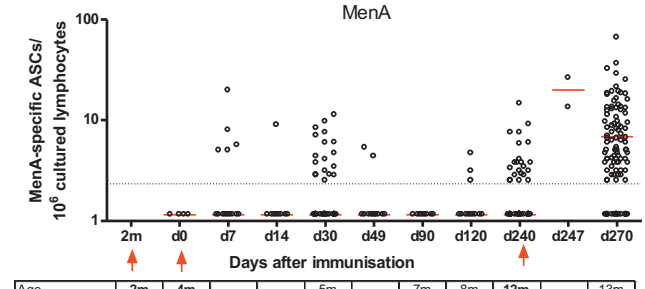
3. Results

3.1. Population

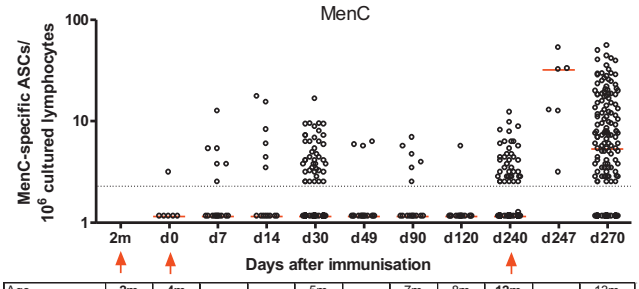
A total of 216 children were enrolled in three groups (108 in group I, 54 in group II and 54 in group III), of whom 196 (91%) completed the study (Table 1).

3.2. Memory B-cell responses

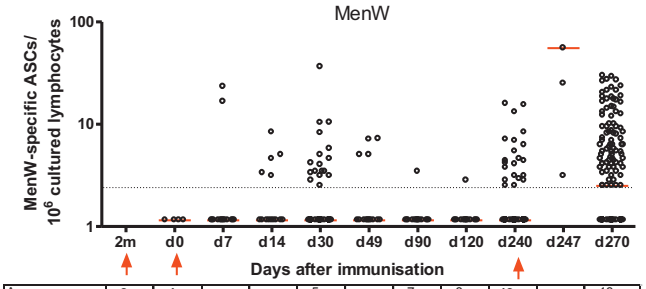
At 5 months of age, the proportion of children who had detectable memory B-cells (i.e. ≥2.5 memory B-cells per million cultured lymphocytes) was 25% for serogroup C and varied between 12–16% for the other polysaccharide serogroups. In contrast, 54% children had detectable CRM_{197}-specific memory B-cells. For all serogroups the median frequency of – memory B-cells was 0 as compared to 3 cells per million cultured lymphocytes for CRM_{197}-memory B-cells. After 5 months, the proportion of children with detectable memory B-cells was similar across all time-points up to and including 12 months of age. At 12 months the proportion of children who had detectable memory B-cells was 24% for serogroup C, varied between 13–17% for the other polysaccharide serogroups, and was 80% for CRM_{197}. Following the booster dose of MenACWY-CRM_{197} at 12 months of age, the proportion of children who had detectable memory B-cells increased and by 13 months was 66% for MenA, 70% for MenC, and around 50% for MenW and MenY, and



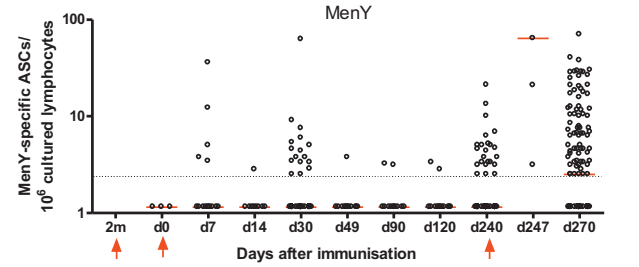
Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number		4	14	9	116	12	8	12	128	2	127
Median		0	0	0	0	0	0	0	0	19.8	4.1
No (%) with detectable cells					18/116				22/128		84/127
					16				17		66



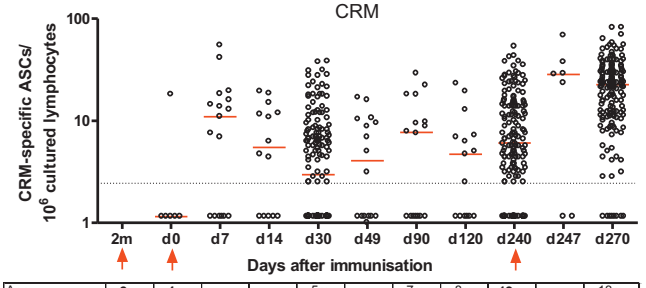
Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number		6	17	14	170	16	16	15	163	7	169
Median		0	0	0	0	0	0	0	0	32.2	5.3
No (%) with detectable cells					42/170				39/163		118/169
					25				24		70



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number		4	15	14	146	15	15	15	161	5	167
Median		0	0	0	0	0	0	0	0	55.3	2.5
No (%) with detectable cells					17/146				21/161		86/167
					12				13		52

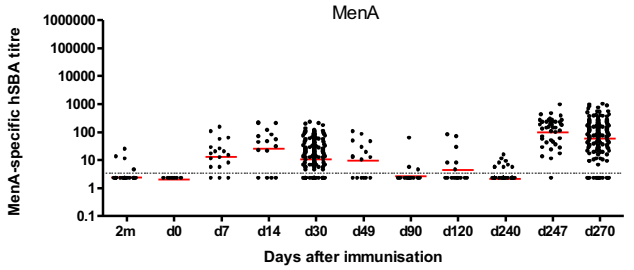


Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number		3	15	9	122	13	12	14	147	5	146
Median		0	0	0	0	0	0	0	0	63.8	2.5
No (%) with detectable cells					16/122				23/147		74/146
					13				16		51

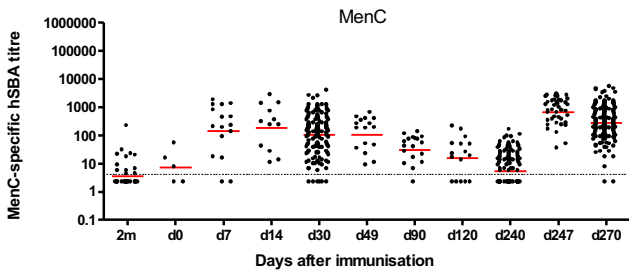


Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number		6	17	14	168	16	16	15	163	7	169
Median		0	10.9	5.5	3	4.1	7.7	4.7	6.1	28.4	22.5
No (%) with detectable cells					98/168				130/163		161/169
					54				80		95

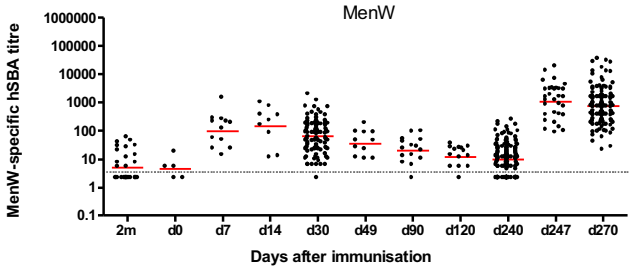
Fig. 1. The memory B-cell response specific to meningococcal-serogroup A, C, W, Y and CRM_{197} was measured following priming with 2 doses of MenACWY-CRM_{197} at 2 and 4 months of age, and following boosting at 12 months of age. The minimum sensitivity of the assay is plotted as a broken line. The zero values have been given the arbitrary value of 1 for illustrative purposes. The horizontal bars represent the median number of specific memory B-cells at each time point for the whole sample size. The tables below each graph report the age, the days following the 4-months immunisation, the number of children tested, the median frequency of memory B-cells, and the number of children with detectable memory B-cells (i.e. ≥2.5 polysaccharide or CRM_{197} specific memory B-cells per million cultured lymphocytes)/total number of children, with the percentage.



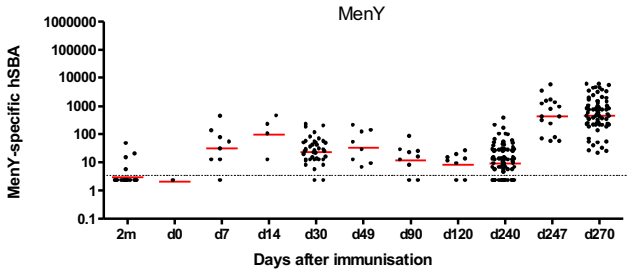
Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	43	7	18	16	179	16	18	16	182	43	187
GMT	2.3	2	13.4	25.6	10.7	9.5	2.6	4.5	2.1	96.6	59.5
No (%) with seroprotection					124/179 69				9/172 5		171/187 91



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	38	5	16	12	165	16	18	16	177	41	176
GMT	3.6	7.2	137.8	180.2	101.7	104.4	30.7	15.3	5.4	656.9	282.2
No (%) with seroprotection					159/165 96				78/177 44		174/176 99



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	29	5	12	9	116	11	15	14	150	31	146
GMT	5.1	4.4	99.2	143.9	62.6	35.3	19.6	12.2	9.5	1063.2	741.1
No (%) with seroprotection					115/116 99				108/150 72		146/146 100



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	21	1	8	4	39	8	9	8	93	16	76
GMT	2.9	2	31.5	95.1	22.9	33.3	11.5	8.2	8.9	435.1	457.1
No (%) with seroprotection					37/39 95				64/93 69		76/76 100

Fig. 2. The functional antibody response measured by serum bactericidal assay using human complement specific to serogroup A, C, W and Y was measured following second dose-priming with MenACWY-CRM$_{197}$ at 4 months of age, and following boosting at 12 months of age. The broken line represents the correlate of protection hSBA ≥4. The horizontal bars represent the geometric mean titre (GMT) at each time point for the whole sample size. The tables below each graph report the age, the days following the 4-months immunisation, the number of children tested, the GMT, and the number of children with seroprotection (hSBA ≥ 1:8)/total number of children, with the percentage.

95% for CRM$_{197}$. The median frequency of polysaccharide-specific memory B-cells at 30 days post-booster varied between 2 and 5 per million cultured lymphocytes and was 23 per million cultured lymphocytes for CRM$_{197}$-memory B-cells (Fig. 1).

3.3. Functional antibody responses (hSBA)

At 5 months of age, one month after 2 dose-priming with MenACWY-CRM$_{197}$ vaccine; the proportion of children who had hSBA titres ≥4 was 69% for MenA, and between 95 and 99% for the other serogroups. Between 5 and 12 months of age, polysaccharide-specific functional antibody titres decreased slowly. At 12 months, the proportion of children who had serogroup specific hSBA titres ≥4 was around 70% for serogroups W and Y, 44% for serogroup C and only 5% for serogroup A. Following the booster dose of MenACWY-CRM$_{197}$ at 12 months of age, the functional antibody titre increased rapidly and at 13 months the proportion of children who had SBA titres ≥4 was 91% for serogroup A and more than 99% for the other serogroups (Fig. 2).

3.4. Antibody responses (IgG)

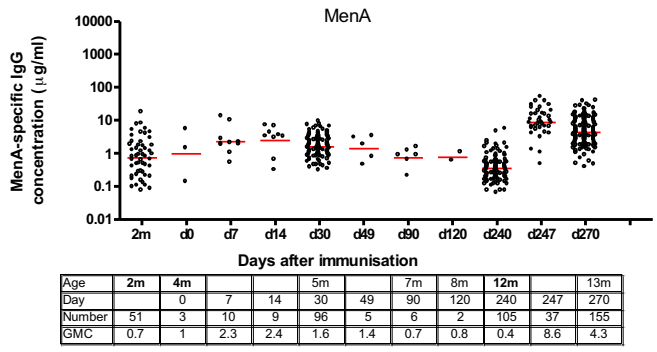
Following the second priming dose of MenACWY-CRM$_{197}$ at 4 months there was a rapid increase in polysaccharide-specific IgG-antibody. Then, the GMC for all serogroups decreased from 5 months until 12 months of age. Following a booster dose of MenACWY-CRM$_{197}$ at 12 months, the concentration of serogroup specific-antibody increased rapidly. For the CRM$_{197}$-IgG concentration there was a clear rise from baseline following the first dose of MenACWY-CRM$_{197}$ at 2 months of age, but no further rise after the second dose of MenACWY-CRM$_{197}$ at 4 months. The concentration of CRM$_{197}$-IgG remained similar from 4 months until 5.5 months and then decreased slowly to 12 months of age. Following the booster dose of MenACWY-CRM$_{197}$ at 12 months of age, the concentration of CRM$_{197}$-IgG increased rapidly (Fig. 3).

3.5. Association between the memory B-cell frequencies at 5 months of age and the hSBA titer/IgG concentration at 12 months and 13 months of age

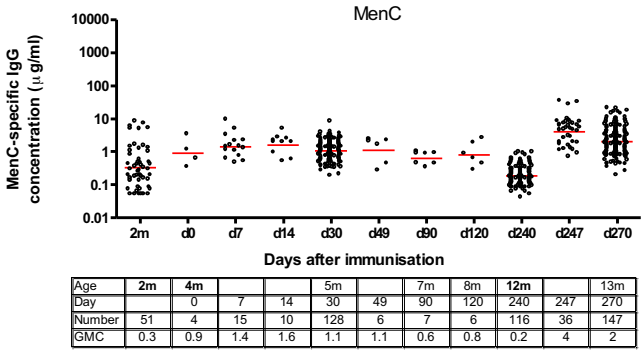
There was a weak inconsistent positive correlation (Spearman's correlation coefficient between 0.2 and 0.4) between memory B-cell frequency measured in peripheral blood at 5 months of age and the antibody measured by hSBA titre and IgG concentration at 12 months and 13 months of age for some serogroups of meningococci (in particular MenC and MenW) (see Table 2). In comparison, there was a Spearman's correlation coefficient of 0.5 ($p < 0.001$, $n = 133$) for the correlation between the frequency of CRM$_{197}$-specific memory B-cells detected at 5 months of age and the CRM$_{197}$-IgG concentration measured at 12 months of age. Furthermore, there was a Spearman correlation coefficient of 0.3 ($p = 0.003$, $n = 134$) for the correlation between CRM$_{197}$-specific memory B-cells at 5 months and the IgG concentration at 13 months.

4. Discussion

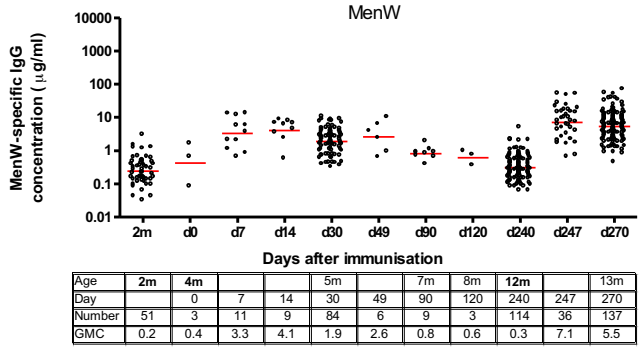
As previously reported, the present study show that there is a good antibody response following MenACWY-CRM$_{197}$ from early



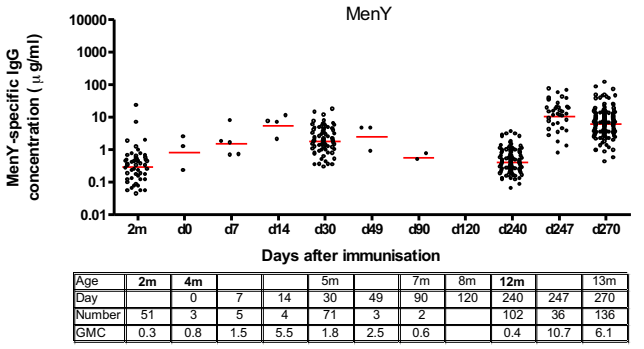
Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	51	3	10	9	96	5	6	2	105	37	155
GMC	0.7	1	2.3	2.4	1.6	1.4	0.7	0.8	0.4	8.6	4.3



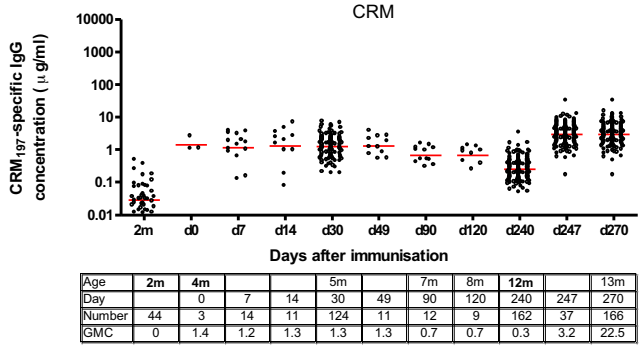
Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	51	4	15	10	128	6	7	6	116	36	147
GMC	0.3	0.9	1.4	1.6	1.1	1.1	0.6	0.8	0.2	4	2



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	51	3	11	9	84	6	9	3	114	36	137
GMC	0.2	0.4	3.3	4.1	1.9	2.6	0.8	0.6	0.3	7.1	5.5



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	51	3	5	4	71	3	2		102	36	136
GMC	0.3	0.8	1.5	5.5	1.8	2.5	0.6		0.4	10.7	6.1



Age	2m	4m			5m		7m	8m	12m		13m
Day		0	7	14	30	49	90	120	240	247	270
Number	44	3	14	11	124	11	12	9	162	37	166
GMC	0	1.4	1.2	1.3	1.3	1.3	0.7	0.7	0.3	3.2	22.5

Fig. 3. The IgG-antibody response specific to serogroup A, C, W, Y and CRM_{197} was measured following second dose-priming with MenACWY-CRM_{197} at 4 months of age, and following boosting at 12 months of age. The horizontal bars represent the geometric mean concentration (GMC) at each time point for the whole sample size. The tables below each graph report the age, the days following the 4-months immunisation, the number of children tested and the GMC.

infancy. However, as it is the case with other conjugate vaccines, this response is associated with rapid waning of antibody in children vaccinated before one year of age, although the response to booster immunisation is excellent [2,11–14]. There appears to be a smaller decline in antibody for children primed with the MenACWY-CRM_{197} at older ages [15]. The rate of decline in polysaccharide-IgG concentration from 5 months to 12 months of age following two-doses-priming with MenACWY-CRM_{197}, is similar to the rate of decline of serogroup C-IgG from 5 months to 12 months of age, following priming with two or three-doses of MenCV in infancy [14,16], and does not appear to depend only on the biological half-life of antibody of 3 weeks [17] as the level at 12 months of age was higher than would be predicted solely by the half-life of antibody. Therefore, the level of persisting antibody by 12 months of age is not only determined by the level reached after primary immunisation at 5 months of age, but also by other determinants such as, presumably, the production of long lived plasma cells and memory B-cells. However, it appears that these mechanisms are not

Table 2
Correlation between polysaccharide and CRM_{197}- specific memory B-cells at 5 months of age with the antibody measured by hSBA titre and IgG concentration at 12 months and 13 months of age.[a]

		hSBA titre at 12 m			hSBA titre at 13 m			IgG at 12 m			IgG at 13 m		
		p	*p*	*n*	*p*	*p*	*n*	*r*	*p*	*n*	*r*	*p*	*n*
MenA	MBc frequency at 5 m	0.09	0.3	104	**0.4**	**0.001**	**105**	−0.08	0.6	56	**0.3**	**0.02**	**89**
MenC	MBc frequency at 5 m	**0.3**	**0.0003**	**147**	**0.2**	**0.02**	**143**	**0.3**	**0.02**	**86**	0.002	1	115
MenW	MBc frequency at 5 m	**0.3**	**0.0005**	**112**	**0.3**	**0.004**	**104**	0.05	0.7	74	**0.2**	**0.02**	**91**
MenY	MBc frequency at 5 m	0.02	0.9	59	0.02	0.9	44	−0.2	0.08	57	**0.3**	**0.03**	**76**
CRM	MBc frequency at 5 m							**0.5**	**<0.001**	**133**	**0.3**	**0.003**	**134**

[a] Pearman's rank correlation coefficient (*r*) with the *p* values (*p*) and the number of subject tested for each association (*n*).

sufficient to maintain serological protection through the ensuing 7 months. The fact that only a small proportion of children produced serogroup-specific memory B-cells after priming, and that there was only a low concentration of IgG-antibodies, in contrast to the high bactericidal antibody response in the short-term followed by a rapid decline by 12 months of age has several possible explanations; The induction of germinal centres with the production of long-lived plasma cells and memory B-cells is very low in early life, as demonstrated in murine studies [18,19], and therefore, antibodies are not maintained in the long-term. Additionally, other murine studies have shown that bone marrow homing of long-lived plasma cells is low in early life [20], a phenomenon that also contributes to the rapid decline of antibody from 5 months until 12 months of age. Alternatively, antigen-specific memory B-cells may be produced at such low frequencies that they cannot be detected by the ELISpot assay used in this study. This small pool of memory B-cells appears to be sufficient to sustain immunological memory (as suggested by the rapid increase in memory B-cells and antibody in peripheral blood following boosting at 12 months), but not to maintain protective antibody in serum. This phenomenon has been observed in a murine study, where the generation of memory B-cells during priming was impaired due to a mutation in the *xid* gene, and the persistence of antibody was reduced, although immunological memory was preserved [21]. The present data raise important issues about the underlying role of memory B-cell, measured in the peripheral blood, in antibody persistence following priming immunisation in early infancy. The aim of this study was to assess whether the memory B-cells detected in peripheral blood following priming immunisation with MenACWY-CRM_{197} at 5 months of age allow prediction of the persistence of antibody by 12 months of age, and the antibody response to booster immunisation with MenACWY-CRM_{197}. In a previous study assessing the antibody and B-cell response after three-dose priming with MenCV at 2, 3, and 4 months of age, 100% of the study children had reached protective bactericidal antibody titres against serogroup C (rSBA $\geq$1:8) at 5 months of age and 64% had detectable MenC-specific memory B-cells [14]. In the same study, it was observed that by 12 months of age only 41% of the study children had maintained protective bactericidal antibody titre, and, there was a good correlation between the memory B-cell responses at 5 months and the persisting antibody titre by 12 months of age (Spearman's correlation coefficient of 0.65, p values = 0.02) [14]. In the present study, there was only a weak positive correlation (Spearman's correlation coefficient of 0.3) between polysaccharide-specific memory B-cell responses at 5 months and polysaccharide-specific persisting antibody at 12 months of age for some serogroups of meningococci (MenC and MenW). These results suggest that the memory B-cell produced at 5 months of age only produce a fraction of the antibody titre at 12 months. The power of these results to detect a correlation is likely to have been limited by the number of individuals in whom no memory B-cells were detected at the 5 months time-point (only 12–25% of infants had detectable memory B-cells depending of the serogroup). In comparison, the proportion of children with detectable carrier-specific memory B-cells was comparable in both studies (54% of CRM_{197}-memory B-cells in the present study, compared to 60% of diphtheria toxoid-memory B-cells in the previous study – at that time CRM_{197} was not available for the ELISpot) [14,22]. In the present study, there was a significant moderate correlation (r = 0.5, $p < 0.001$) between the carrier-specific memory B-cells detected at 5 months of age and the persistence of antibody at 12 months of age which may imply that the assay in the present study was not sensitive enough to test the correlations between the lower memory B-cell responses at 5 months of age and the persisting antibody at 12 months of age for polysaccharide antigens. The lower proportion of children with detectable MenC-memory B-cells in the present study in comparison to the preceding study [14,22] has several possible explanations. The children were primed with two doses of MenACWY-CRM_{197}, in comparison to three doses of MenCV in the preceding study (because the new UK vaccination schedule includes only two doses of MenC-conjugate vaccine for primary immunization). Furthermore, MenACWY-CRM_{197} vaccine is somewhat less immunogenic than the MenCV for the serogroup C meningococcal antibody response when studied in infants from 2 months of age [2], presumably because the MenACWY-CRM_{197} contains a lower concentration of serogroup C capsular saccharide in comparison to the MenCV, is not adjuvanted with aluminium phosphate and is a multivalent vaccine with possible interference between the conjugates. However, another study comparing the functional antibody response following a single dose of MenCV or MenACWY-CRM_{197} at 12 months of age found similar results for serogroup C meningococcal antibody response [23]. At the level of the individual, for the MenACWY-CRM_{197}, the current memory B-cell assay was insufficiently sensitive to detect memory B-cells on enough individuals to be a reliable predictor of antibody persistence in all individuals. The assay sensitivity could have been increased by culturing larger numbers of cells for each antigen but this would have meant a reduction in the antigens assessed. For antigens where more B-cells were detected (CRM_{197} and MenC) the correlations with IgG and hSBA at 12 months suggest the usefulness of this assay. The fact that the memory B cell-immune response was of greater magnitude for the carrier-protein than the polysaccharide antigen can be explained by previous exposure (both cumulative dose and number of doses) to the protein-carrier antigen through the use of other protein-polysaccharide conjugate vaccines using CRM_{197} as carrier protein and as a result of exposure to diphtheria toxoid vaccine, from which CRM_{197} is derived. In contrast for MenC-specific memory B cells, the increase in the memory B cell frequency may be partly explained by the priority given to this antigen in the conduct of the study where there were low volume blood samples, which increased the sensitivity of the ELISpot assay for MenC in comparison to the other serogroups. There was no difference for the bactericidal antibody response between serogroups C, W and Y. In conclusion, the MenACWY-CRM_{197} is immunogenic in early infancy, although few anticapsular-specific memory B-cells and low production of IgG-antibody were detected after two doses-priming with this vaccine. However these antibodies were strongly bactericidal and likely to provide protection. Despite there being a relatively low-frequency of memory B-cells detected after priming these were associated with a potent antibody responses after a booster dose of MenACWY-CRM_{197} at a year of age, together with an expansion of memory B-cell responses, which may indicate strong priming or simply the improved responses that are possible with maturation of the immune system by a year of age [14,24,25]. Whilst comparison with other studies indicates that memory B-cell assays are a useful method of assessing immunological memory, the low numbers of B-cells detected here suggest that this method may not be suitable for all antigens, especially in young children where only small volumes of blood can be obtained for analysis, thus limiting sensitivity. For the moment the only way to guarantee long-term immunity in the population is by sustaining immunity through the use of booster doses of vaccine. Recent data suggest that boosters in the second year of life will raise the bactericidal antibody temporarily [26] but more sustained responses are achieved when booster doses are administered later in childhood (after 6 years of age) [27]. Boosters administered in adolescence, as has recently been recommended by the UK Department of Health's Joint Committee on Vaccination and Immunisation [28], are likely to provide more sustained immunity and also to maintain herd protection since this age group has high colonisation rates and is thought to be responsible for driving transmission of serogroup C meningococci [29].

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Conflicts of interest: AJP has conducted clinical trials on behalf of Oxford University, sponsored by Wyeth/Pfizer Vaccines, GlaxoSmithKline Vaccines, Sanofi Pasteur, Sanofi Pasteur MSD and Novartis Vaccines, but does not accept any personal payments from vaccine manufacturers. Educational grants for organisation of scientific meetings are paid to an educational/administrative fund held by the Department of Paediatrics, University of Oxford. DFK has received assistance to attend scientific meetings from Wyeth Vaccines and GSK. MDS has received assistance to attend scientific meetings from Wyeth Vaccines, Novartis Vaccines and GlaxoSmithKline Vaccines and has had travel and accommodation expenses paid by Novartis Vaccines while working in collaboration with Novartis Vaccines in Siena, Italy. T.O. is an employee of Novartis Vaccines.

References

[1] Perrett KP, Snape MD, Ford KJ, John TM, Yu LM, Langley JM, et al. Immunogenicity and immune memory of a nonadjuvanted quadrivalent meningococcal glycoconjugate vaccine in infants. Pediatric Infect Dis J 2009;28(March (3)):186–93.
[2] Snape MD, Perrett KP, Ford KJ, John TM, Pace D, Yu LM, et al. Immunogenicity of a tetravalent meningococcal glycoconjugate vaccine in infants: a randomized controlled trial. JAMA 2008;299(January (2)):173–84.
[3] Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129(June (6)):1307–26.
[4] Fothergill. Influenzal meningitidis: the relation of age incidence to the bactericidal power of blood against causal organism. J Immunol 1933;24:273–84.
[5] Manz RA, Lohning M, Cassese G, Thiel A, Radbruch A. Survival of long-lived plasma cells is independent of antigen. Int Immunol 1998;10(November (11)):1703–11.
[6] Smith KG, Hewitson TD, Nossal GJ, Tarlinton DM. The phenotype and fate of the antibody-forming cells of the splenic foci. Eur J Immunol 1996;26(February (2)):444–8.
[7] Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity 1998;8(March (3)):363–72.
[8] Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, et al. Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. The Multilaboratory Study Group. Clin Diagn Lab Immunol 1997;4(March (2)):156–67.
[9] Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. Infect Immun 2001;69(March (3)): 1568–73.
[10] 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(June (6)):1475–82.
[11] Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. Lancet 2004;364(July (9431)):365–7.
[12] MacLennan JM, Shackley F, Heath PT, Deeks JJ, Flamank C, Herbert M, et al. Safety, immunogenicity, and induction of immunologic memory by a serogroup C meningococcal conjugate vaccine in infants: a randomized controlled trial. JAMA 2000;283(June (21)):2795–801.
[13] Snape MD, Kelly DF, Green B, Moxon ER, Borrow R, Pollard AJ. Lack of serum bactericidal activity in preschool children two years after a single dose of serogroup C meningococcal polysaccharide-protein conjugate vaccine. Pediatric Infect Dis J 2005;24(February (2)):128–31.
[14] Blanchard Rohner G, Snape MD, Kelly DF, John T, Morant A, Yu LM, et al. The magnitude of the antibody and memory B cell responses during priming with a protein-polysaccharide conjugate vaccine in human infants is associated with the persistence of antibody and the intensity of booster response. J Immunol 2008;180(February (4)):2165–73.
[15] Black S, Klein NP, Shah J, Bedell L, Karsten A, Dull PM. Immunogenicity and tolerability of a quadrivalent meningococcal glycoconjugate vaccine in children 2–10 years of age. Vaccine 2010;28(January (3)):657–63.
[16] Borrow R, Andrews N, Findlow H, Waight P, Southern J, Crowley-Luke A, et al. Kinetics of antibody persistence following administration of a combination meningococcal serogroup C and haemophilus influenzae type b conjugate vaccine in healthy infants in the United Kingdom primed with a monovalent meningococcal serogroup C vaccine. Clin Vaccine Immunol 2010;17(January (1)):154–9.
[17] Vieira P, Rajewsky K. The half-lives of serum immunoglobulins in adult mice. Eur J Immunol 1988;18(February (2)):313–6.
[18] 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(March (6)):2824–32.
[19] Marshall-Clarke S, Reen D, Tasker L, Hassan J. Neonatal immunity: how well has it grown up? Immunol Today 2000;21(January (1)):35–41.
[20] Pihlgren M, Schallert N, Tougne C, Bozzotti P, Kovarik J, Fulurija A, et al. Delayed and deficient establishment of the long-term bone marrow plasma cell pool during early life. Eur J Immunol 2001;31(March (3)):939–46.
[21] Ridderstad A, Nossal GJ, Tarlinton DM. The xid mutation diminishes memory B cell generation but does not affect somatic hypermutation and selection. J Immunol 1996;157(October (8)):3357–65.
[22] Kelly DF, Snape MD, Perrett KP, Clutterbuck EA, Lewis S, Blanchard Rohner G, et al. Plasma and memory B-cell kinetics in infants following a primary schedule of CRM 197-conjugated serogroup C meningococcal polysaccharide vaccine. Immunology 2009;127(May (1)):134–43.
[23] Halperin SA, Diaz-Mitoma F, Dull P, Anemona A, Ceddia F. Safety and immunogenicity of an investigational quadrivalent meningococcal conjugate vaccine after one or two doses given to infants and toddlers. Eur J Clin Microbiol Infect Dis 2010;29(March (3)):259–67.
[24] Siegrist CA, Aspinall R. B-cell responses to vaccination at the extremes of age. Nat Rev 2009;9(March (3)):185–94.
[25] Kelly DF, Snape M, Clutterbuck EC, Green S, Snowden C, Diggle L, et al. CRM197-conjugated serogroup C meningococcal capsular polysaccharide, but not the native polysaccharide, induces persistent antigen specific memory B cells. Blood 2006 May.
[26] Khatami A, Snape MD, Wysocki J, John TM, Westcar S, Mesaros N, et al. Persistence of antibody response following a booster dose of Hib-MenC-TT glycoconjugate vaccine to five years: a follow-up study. Pediatric Infect Dis J 2012;31(October (10)):1069–73.
[27] Perrett KP, Winter AP, Kibwana E, Jin C, John TM, Yu LM, et al. Antibody persistence after serogroup C meningococcal conjugate immunization of United Kingdom primary-school children in 1999–2000 and response to a booster: a phase 4 clinical trial. Clin Infect Dis 2010;50(12):1601–10. Jun 15.
[28] Department of Health. Joint Committee on Vaccination and Immunisation MotmoWOL. Joint Committee on Vaccination and Immunisation, Minute of the meeting on Wednesday 3 October 2012 London; 2012.
[29] Pollard AJ, Perrett KP, Beverley PC. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nat Rev 2009;9(March (3)):213–20.