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The influence of neonatal Bacille Calmette-Guérin (BCG) immunisation on heterologous vaccine responses in infants

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ABSTRACT

Introduction: Bacillus Calmette-Guérin vaccine (BCG), one of the most widely used vaccines, does not only provide protection against tuberculosis and other mycobacterial infections, but also has non-specific (heterologous) immunomodulatory effects. In participants in a randomised trial, we investigated the effect of neonatal BCG immunisation on antibody responses to routine infant vaccines given in the first year of life.

Methods: Antibodies against antigens in the diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b (Hib), and the 13-valent pneumococcal conjugate vaccines were measured in 91 (45 BCG-vaccinated, 46 BCG-naïve) infants one month after, and in 310 (169 BCG-vaccinated, 141 BCG-naïve) infants seven months after immunisation at 6 weeks, 4 and 6 months of age. In addition, antibodies against meningococcus C, Hib, measles, mumps, and rubella were measured in 147 (78 BCG-vaccinated, 69 BCG-naïve) infants one month after immunisation at 12 months of age. The seroprotection rates for each vaccine and the geometric mean concentrations (GMC) of antibodies were compared in BCG-vaccinated and BCG-naïve infants.

Results: At 7 months of age, seroprotection rates were high in both BCG-vaccinated and BCG-naïve infants. At 13 months of age, seroprotection rates were lower than at 7 months of age, particularly for pertussis and a number of pneumococcal antigens, with generally higher rates for the latter in BCG-vaccinated infants. Although not statistically significant, antibody responses in BCG-vaccinated infants were consistently higher against diphtheria, tetanus, and pneumococcal antigens at both 7 and 13 months of age, and against measles and mumps at 13 months of age, but were lower against Hib one month after immunisation at both 7 and 13 months of age.

Conclusion: The immunomodulatory effect of BCG on antibody responses to heterologous vaccines adds to the evidence that BCG immunisation at birth has broad heterologous effects on the infant immune system.

1. Introduction

Bacillus Calmette-Guérin (BCG) vaccine, is given to over 160 million infants each year, making it one of the most widely used vaccines worldwide. The vaccine is primarily considered to protect

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against tuberculosis (TB), particularly infant TB [1,2] and other mycobacterial infections [3]. However, BCG has powerful non-specific (heterologous) immunomodulatory effects, which are of particular importance for the early development of the immune system [4,5]. Randomised trials have shown that BCG reduces childhood mortality from causes other than TB by approximately one quarter in developed countries [6] and by approximately half in high-mortality settings [7–9]. The reduced mortality is attributed to decreased deaths from pneumonia, sepsis and diarrhoea [7,10,11]. Additionally, BCG immunisation is reported to decrease hospitalisation rates due to non-TB respiratory infections and sepsis [12,13]. Moreover, the immunomodulatory effects of BCG might decrease the risk for atopic dermatitis [14] and allergies [15]; are proposed to beneficial in the treatment of multiple sclerosis [16] and diabetes mellitus [17]; and are used in the prevention and treatment of bladder cancer [18], melanoma [19], and leukaemia [20].

The immunomodulatory effects of BCG also influence responses to heterologous immunisations [21]. Previous or concurrent administration of BCG with other vaccines has been associated with significantly higher concentrations of antibodies against hepatitis B (HepB), polio, pneumococcus and influenza [22–26], but not with altered antibody responses to diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b (Hib) and typhoid [23,24,27,28]. One study reported lower antibodies concentrations against HepB in infants who were vaccinated with BCG compared to BCG-naïve infants [23].

Using samples from participants in a randomised trial, we investigated the effect of BCG immunisation at birth on humoral responses to routine infant immunisations given in the first year of life.

2. Methods

2.1. Study design and population

Participants were a subset of infants from The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR). This randomised trial comprises 1272 healthy infants recruited antenatally from 2013 to 2016 to investigate whether BCG immunisation at birth protects against childhood infection, allergy and asthma. Inclusion criteria were: greater than 32 weeks gestation, birth weight greater than 1500 g, mother not HIV positive, absence of symptoms or signs of illness, and no known contact with TB. Twins of participants were excluded, as they were not considered independent. Three-monthly parent questionnaires were used to prospectively collect demographic and other data.

2.2. Intervention

Infants in MIS BAIR were randomised within 10 days of birth to either receive BCG-Denmark (Statens Serum Institut, Copenhagen) 0.05 ml intradermally in the deltoid region of the left arm (BCG-vaccinated) or no BCG (BCG-naïve). The infants included in this study were not randomised specifically for the measurement of vaccine responses, but were a subset of the randomised participants.

2.3. Routine immunisation

All infants received routine immunisations according to the Australian National Immunisation Program: at birth: intramuscular HepB vaccine (H-B-Vax II Paediatric® (*bioCSL*)); at 6 weeks, 4 months and 6 months of age: intramuscular combined diphtheria-tetanus-acellular pertussis (DTPa)-HepB-inactivated polio (IPV)-Hib vaccine (Infanrix® hexa (*GlaxoSmithKline*)), intramuscular 13-valent conjugate pneumococcal vaccine (PCV13) (Prevenar13® (*Wyeth*)), and oral rotavirus vaccine (RotaTeq® (*Merck*)); at 12 months of age: subcutaneous measles-mumps-rubella (MMR) vaccine (Priorix® (*GlaxoSmithKline*)) and intramuscular combined meningococcal C (MenC) and Hib vaccine (Menitorix® (*GlaxoSmithKline*)). Vaccine records were obtained from individual immunisation records and/or the Australian Immunisation Register.

2.4. Blood collection

From the subset of participants whose parent/guardian provided consent, blood samples were collected at study visits one month after the scheduled 6-month routine vaccines ('7 months') and the scheduled 12-month routine vaccines ('13 months') in tubes containing sodium-heparin (S-monovette® (*Sarstedt*)). Only participants who had a blood sample taken 28 ± 14 days after their 6-month and/or 12-month routine vaccinations were included in the analysis of immediate post-vaccination responses. Plasma was stored at −80 °C until analysis.

2.5. Antibody measurement

Plasma samples were analysed at the National Institute for Health and Environment, in Bilthoven, the Netherlands. Immunoglobulin (Ig) G antibodies were measured using fluorescent bead-based multiplex immune-assays (Luminex xMAP technology) [29–34] against 26 vaccine antigens: (diphtheria, tetanus, pertussis (pertussis toxin (PT), filamentous haemagglutinin (FHA), pertactin (PRN)), polio (types 1, 2, 3), Hib, pneumococcus (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F), meningococcus type C (MenC), measles, mumps and rubella). In all assays, international or in-house reference controls and blanks were included on each plate. All analyses were performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).

2.6. Statistical analysis

The proportion of infants with an antibody concentration above the standard protective correlate value for each vaccine was calculated (seroprotection rate) for the BCG-vaccinated and BCG-naïve groups [32,35,36]. The Clopper-Pearson method was used to estimate the 95% confidence intervals (CIs) of the seroprotection rates. For FHA and PRN, no accepted protective correlate value exists. Proportions were compared using Fisher's exact test and 95% confidence interval (CI) for differences in proportions estimated. In each group, the geometric mean concentration (GMC) for each vaccine antibody (IgG) was calculated. Geometric mean ratios (GMR) with 95% CI were derived from the anti-logged coefficient using a linear regression with log-concentrations as outcome and BCG immunisation status as covariate. Effect modification was assessed using the following pre-specified factors: maternal BCG immunisation status, maternal dTpa (*Boostrix*® (GlaxoSmithKline)) immunisation in pregnancy, gestational age, delivery mode, sex, birth weight, age at randomisation (pre-specified as < 48 h versus ≥ 48 h), age at first DTPa-HepB-IPV-Hib/PCV13 vaccination, MMR immunisation status (for responses at 13 months of age), age at blood sampling and time between 6-month or 12-month immunisation and blood sampling. The GMR for each vaccine antigen was adjusted for those factors that both differed between groups and had an effect on that vaccine antigen response. As the Hib-MenC vaccine includes a tetanus toxoid (as carrier protein) and a Hib component, infants who had received this vaccine before blood sampling at 13 months of age were excluded from the

analysis of persistence of antibodies against these two vaccine antigens. A 5%-significance level was used. All statistical analyses were done using R version 3.4.3.

2.7. Ethics

Informed consent was obtained from participants' parents or legal guardians. The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC, authorisation (33025). MIS BAIR is registered with the Australia & New Zealand Clinical Trials Registry (1051228) and the U.S. National Institutes of Health (NCT01906853).

3. Results

Of the 471 potentially eligible participants, seven were excluded because they were a twin of another participant, four because they were not vaccinated according to the standard schedule, one because he received BCG outside the study and one because vaccine records were not available (Fig. 1). Of the remaining participants, 90 were excluded because their blood samples were not taken 28 ± 14 days after their 6-month and 12-month routine vaccines. For the final analysis, at 7 months of age, 91 (45 BCG-vaccinated and 46 BCG-naïve) participants were included for measurement of antibodies following their primary course of vaccines ending at 6 months of age. At 13 months of age, 147 (78 BCG-vaccinated and 69 BCG-naïve) were included for measurement of antibodies following their 12-month vaccines. In addition, at 13 months of age, a total of 310 (169 BCG-vaccinated and 141 BCG-naïve) participants had their samples analysed for persistence of antibodies following their primary course of vaccines. For these participants, blood was taken 7 months ± 23 days after their 6-month vaccinations. Baseline characteristics of participants are summarised in Table 1.

3.1. The effect of BCG immunisation on seroprotection rates

One month after immunisation, the large majority of infants had antibody concentrations above the standard protective correlate, except for Hib and pneumococcus serotype 4 at 7 months of age, and mumps at 13 months of age. In contrast, at 13 months of age, for immunisations given in the first 6 months, a high proportion of infants did not have antibody concentrations above the protective correlate, particularly for PT and pneumococcus serotype 4 (Table 2). There was no consistent difference in seroprotection rates between BCG-vaccinated and BCG-naïve infants at either 7 or 13 months of age. Although there was a suggestion of higher seroprotection rates in BCG-vaccinated infants for some

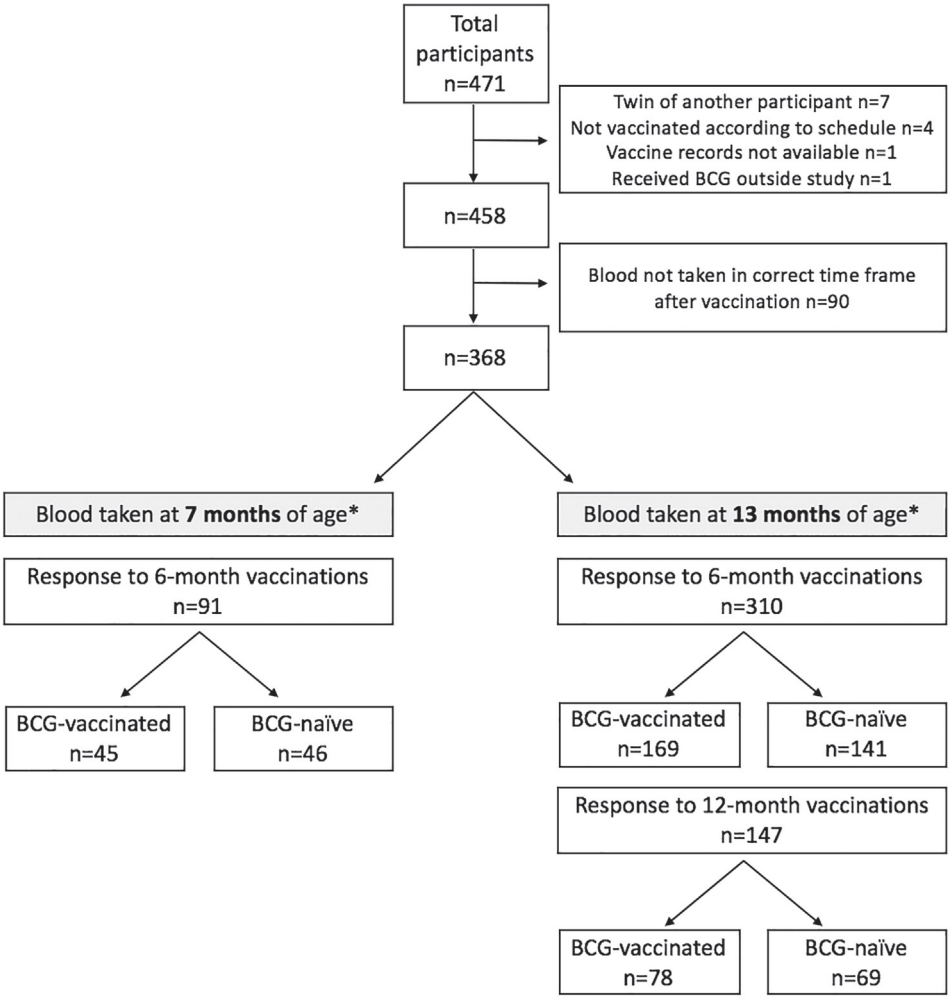

Fig. 1. Selection of participants.

Table 1
Characteristics of participants.

	Samples at 7 months of age for antibodies to primary course of vaccines ending at 6 months of age			Samples at 13 months of age for persistence of antibodies to primary course of vaccines ending at 6 months of age			Samples at 13 months of age for antibodies to 12-month vaccines		
	BCG-vaccinated (n = 45) n (%) or median (IQR)	BCG-naïve (n = 46) n (%) or median (IQR)	p-value	BCG-vaccinated (n = 169) n (%) or median (IQR)	BCG-naïve (n = 141) n (%) or median (IQR)	p-value	BCG-vaccinated (n = 78) n (%) or median (IQR)	BCG-naïve (n = 69) n (%) or median (IQR)	p-value
Mother BCG-vaccinated	13 (30)[1]	9 (21)[2]	0.48	35 (22)[3]	30 (22)[4]	0.92	22 (29)[5]	12 (18)[2]	0.24
Maternal dTpa in pregnancy	18 (40)	28 (61)	0.25	85 (50)	61 (43)	0.46	39 (50)	30 (43)	0.63
Gestational age (weeks)	39.1 (38.5–40.4)	40.0 (38.7–40.5)	0.46	39.4 (38.5–40.3)	39.2 (38.3–40.3)	0.25	39.4 (38.5–40.5)	39.2 (38.3–40.3)	0.23
Caesarean section	21 (47)	14 (30)	0.29	62 (37)	55 (39)	0.78	27 (35)	27 (39)	0.70
Sex (male)	20 (44)	23 (50)	0.75	79 (47)	72 (51)	0.66	36 (46)	36 (52)	0.67
Birth weight (kg)	3.44 (3.06–3.62)	3.49 (3.22–3.81)	0.44	3.46 (3.14–3.79)	3.42 (3.12–3.75)	0.27	3.45 (3.13–3.68)	3.36 (3.14–3.64)	0.48
Age at randomisation (hours)	33.5 (20.4–53.7)	31.1 (19.6–58.1)	0.87	26.2 (17.3–51.1)	25.6 (19.3–56.3)	0.31	24.9 (16.9–47.8)	26.5 (19.6–50.8)	0.32
MMR/Hib-MenC-vaccinated	–	–		110 (65)	110 (71)	0.30	78 (1 0 0)	69 (1 0 0)	1
Age at routine immunisation (days)									
– 6-week vaccines	44 (42–48)	45 (43–47)[6]	0.94	46 (43–50)	45 (43–49)	0.94	45 (43–48)	45 (43–48)	0.77
– 4-month vaccines	123 (118–128)	123 (115–126)	0.16	125 (120–133)	124 (121–131)	0.12	124 (119–132)	124 (122–130)	0.71
– 6-month vaccines	188 (183–194)	186 (178–193)	0.12	191 (185–208)	191 (184–200)	0.07	190 (184–207)	190 (185–197)	0.14
– 12-month vaccines	–	–		377 (371–385)	374 (368–381)	0.37	377 (370–385)	374 (368–381)	0.09
Interval between (days)									
– 6-month vaccines to 7-month blood sample	30 (24–36)	26 (20–38)	0.40	–	–		–	–	
– 6-month vaccines to 13-month blood sample	–	–		209 (189–223)	210 (191–227)	0.05	–	–	
– 12-month vaccines to 13-month blood sample	–	–		–	–		30 (24–35)	27 (21–37)	0.45
Age at blood sampling (days)	222 (210–227)	216 (206–226)	0.05	402 (386–416)	400 (389–418)	0.24	409 (399–415)	402 (394–412)	0.07

p-values were determined by *t*-test for continuous variables and Pearson's Chi-squared test for categorical variables.
BCG = Bacille Calmette-Guérin vaccine, dTpa = diphtheria-tetanus-acellular pertussis vaccine.
[1] 1 mother with unknown BCG status.
[2] 3 mothers with unknown BCG status.
[3] 7 mothers with unknown BCG status.
[4] 6 mothers with unknown BCG status.
[5] 2 mothers with unknown BCG status.
[6] Date not available for 1 participant.

Table 2
Seroprotection rates in participants at 7 and 13 months of age.

Vaccine antigen	Protective correlate	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age				Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age			
		BCG-vaccinated (n = 45) % (n); (95% CI)	BCG-naïve (n = 46) % (n); (95% CI)	Difference % (95% CI)	p-value	BCG-vaccinated (n = 169) % (n); (95% CI)	BCG-naïve (n = 141) % (n); (95% CI)	Difference % (95% CI)	p-value
Diphtheria	0.1 IU/mL[1]	100 (45); (92.1, 100)	93.5 (43); (82.1, 98.6)	6.5 (−1.7, 17.6)	0.24	98.8 (167); (95.6, 99.9)	97.9 (138); (93.9, 99.6)	0.9 (−2.3, 5.0)	0.66
Tetanus	0.1 IU/mL[1]	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (93); (96.1, 100)[2]	100 (73); (95.0, 100)[2]	0 (−4.0, 5.0)[2]	–
PT	25 IU/mL	88.9 (40); (75.9, 96.3)	91.3 (42); (79.2, 97.8)	−2.4 (−16.2, 11.0)	0.74	30.2 (51); (23.4, 37.7)	30.5 (43); (23.0, 38.8)	−0.3 (−10.7, 9.9)	1
Hib	0.15 μg/mL	71.1 (32); (55.7, 83.6)	80.4 (37); (66.1, 90.6)	−9.3 (−26.9, 8.5)	0.34	73.1 (68); (62.9, 81.8)[2]	80.8 (59); (70.0, 89.1)[2]	−7.7 (−20.3, 5.5)[2]	0.27
Polio type 1	0.23 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	100 (141); (97.4, 100)	0 (−2.2, 2.6)	–
Polio type 2	0.29 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	99.4 (168); (96.7, 100)	100 (141); (97.4, 100)	−0.6 (−3.3, 2.1)	1
Polio type 3	0.12 IU/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	100 (141); (97.4, 100)	0 (−2.2, 2.6)	–
Pn 1	0.35 μg/mL	97.8 (44); (88.2, 99.9)	95.7 (44); (85.2, 99.5)	2.0 (−7.8, 12.7)	1	92.3 (156); (87.2, 95.8)	95.0 (134); (90.0, 98.0)	−2.7 (−8.5, 3.1)	0.36
Pn 3	0.35 μg/mL	97.8 (44); (88.2, 99.9)	100 (46); (92.2, 100)	−2.2 (−11.6, 5.7)	0.49	71.6 (121); (64.2, 78.3)	67.4 (95); (59.0, 75.0)	4.2 (−6.0, 14.6)	0.46
Pn 4	0.35 μg/mL	77.8 (35); (62.9, 88.8)	60.9 (28); (45.4, 74.9)	16.9 (−2.2, 35.0)	0.11	26.0 (44); (19.6, 33.3)	19.9 (28); (13.6, 27.4)	6.2 (−3.3, 15.4)	0.23
Pn 5	0.35 μg/mL	97.8 (44); (88.2, 99.9)	97.8 (45); (88.5, 99.9)	0 (−9.7, 9.4)	1	87.0 (147); (81.0, 91.7)	87.2 (123); (80.6, 92.3)	−0.3 (−7.8, 7.6)	1
Pn 6A	0.35 μg/mL	100 (45); (0.92, 100)	95.7 (44); (0.85, 99.5)	4.3 (−3.8, 14.6)	0.49	87.6 (148); (81.6, 92.1)	85.1 (85); (78.1, 90.5)	2.5 (−5.2, 10.5)	0.62
Pn 6B	0.35 μg/mL	91.1 (41); (78.8, 97.5)	87.0 (40); (73.7, 95.1)	4.2 (−9.7, 18.3)	0.74	67.5 (114); (59.8, 74.5)	60.3 (120); (51.7, 68.4)	7.2 (−3.5, 17.9)	0.19
Pn 7F	0.35 μg/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	100 (169); (97.8, 100)	98.6 (139); (95.0, 99.8)	1.4 (−0.8, 5.0)	0.21
Pn 9V	0.35 μg/mL	100 (45); (92.1, 100)	95.7 (44); (85.2, 99.5)	4.3 (−3.8, 14.6)	0.49	78.1 (132); (71.1, 84.1)	73.0 (103); (64.9, 80.2)	5.1 (−4.5, 14.8)	0.35
Pn 14	0.35 μg/mL	91.1 (41); (78.8, 97.5)	91.3 (42); (79.2, 97.6)	−0.2 (−13.4, 12.9)	1	86.4 (146); (80.3, 91.2)	86.5 (122); (79.8, 91.7)	−0.1 (−7.8, 7.8)	1
Pn 18C	0.35 μg/mL	97.8 (44); (88.2, 99.9)	93.5 (43); (82.1, 98.6)	4.3 (−5.9, 15.7)	0.62	75.7 (128); (68.6, 82.0)	78.0 (110); (70.3, 84.5)	−2.3 (−11.6, 7.3)	0.69
Pn 19A	0.35 μg/mL	95.6 (43); (84.9, 99.5)	91.3 (42); (79.2, 97.6)	4.3 (−7.4, 16.7)	0.68	53.3 (90); (45.4, 61.0)	46.1 (65); (37.7, 54.7)	7.2 (−4.0, 18.2)	0.25
Pn 19F	0.35 μg/mL	100 (45); (92.1, 100)	100 (46); (92.2, 100)	0 (−7.9, 7.8)	–	98.8 (167); (95.8, 99.9)	100 (141); (97.4, 100)	−1.2 (−4.2, 1.5)	0.50
Pn 23F	0.35 μg/mL	95.6 (43); (84.9, 99.5)	91.3 (42); (79.2, 97.6)	4.3 (−7.4, 16.7)	0.68	64.5 (109); (56.8, 71.7)	57.4 (81); (48.8, 65.7)	7.1 (−3.8, 17.8)	0.24
						Antibodies to 12-month vaccines measured at 13 months of age			
						BCG-vaccinated (n = 78) % (n); (95% CI)	BCG-naïve (n = 69) % (n); (95% CI)	Difference % (95% CI)	p-value
Measles	0.12 IU/mL					98.7 (77); (93.1, 100)	97.1 (67); (89.9, 99.6)	1.6 (−4.4, 8.8)	0.60
Mumps	45 IU/mL					65.4 (51); (53.8, 75.8)	56.5 (39); (44.0, 68.4)	8.9 (−6.9, 24.4)	0.31
Rubella	10 IU/mL					88.5 (69); (79.2, 94.6)	85.5 (59); (75.0, 92.8)	3.0 (−8.2, 14.7)	0.66
MenC	2 μg/mL					98.7 (77); (93.1, 100)	98.6 (68); (92.2, 100)	0.2 (−5.6, 6.6)	1
Hib	0.15 μg/mL					98.7 (77); (92.1, 100)	98.6 (68); (92.2, 100)	0.2 (−5.6, 6.6)	1
Tetanus	0.01 IU/mL					93.6 (73); (85.7, 97.9)	95.7 (66); (87.8, 99.1)	−2.1 (−10.5, 6.5)	0.72

BCG = Bacille Calmette-Guérin vaccine, CI = confidence interval, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PT = pertussis toxin.
[1] 0.01 IU/mL at 13 months of age.
[2] Only participants who had not had Hib-MenC.

pneumococcal serotypes at 7 months of age and for mumps and rubella at 13 months of age, these differences were not statistically significant (Table 2, Fig. 2).

3.2. The effect of BCG immunisation on concentration of antibodies to the primary course of vaccines

GMCs for antibodies to immunisations given in the first 6 months at 7 months of age and their persistence at 13 months of age are shown in Tables 3 and Supplementary Data, Figs. 1–2. After adjusting for pre-specified factors, GMRs were consistently above one, indicating higher average antibody responses in BCG-vaccinated infants, for diphtheria, tetanus and pneumococcal antigens at both 7 months and 13 months, though these findings were not statistically significant. In contrast, for pertussis and polio, there was no consistent direction of change at both time points: GMRs for pertussis antigens were mostly above one at both time points; GMRs for polio antigens were below one for all three antigens at 13 months of age (Table 3, Fig. 3).

3.3. The effect of BCG immunisation on concentration of antibodies to the 12-month vaccines

GMCs at 13 months of age for antibodies to immunisations given at 12 months of age in BCG-vaccinated and BCG-naïve infants are shown in Table 3 and Supplementary Data Fig. 3. After adjustment for pre-specified factors, GMRs were above one in BCG-vaccinated infants for measles and mumps (Table 3, Fig. 3). In contrast, the GMR for Hib at 13 months of age indicated a lower response to this booster dose in BCG-vaccinated infants, consistent with the finding at 7 months of age of a diminished response to the primary course of Hib immunisations. These findings did not, however, reach statistically significance.

3.4. Effect modifiers

Age at bleeding, time between immunisation and bleeding, and maternal dTpa immunisation during pregnancy were the strongest effect modifiers of infant vaccine responses. We also observed a moderate effect of biological sex and gestational age on vaccine responses, while maternal BCG status, age at randomisation, birth weight and delivery mode had little effect.

3.5. Effect modification by 'age at randomisation'

Seroprotection rates and GMCs/GMRs of infants randomised on day 0–1 of life and those randomised on day 2–10 of life are shown in Supplementary Data Tables 1–4 and in Supplementary Data Fig. 4. In infants randomised on day 2–10, at 7 months of age, BCG-vaccinated infants had significantly higher antibody responses to pneumococcal serotype 9V, 19F and 23F compared with BCG-naïve infants (Supplementary Data Table 4). In infants randomised on day 0–1, at 13 months of age, BCG-vaccinated infants had significantly higher antibody responses (persistence of antibodies) against tetanus compared with BCG-naïve infants (Supplementary Data Table 2).

Within the group of infants who were BCG-vaccinated, at 7 months of age, antibody concentrations against all 22 vaccine antigens were higher in those randomised to BCG on day 2–10 of life compared to those randomised on day 0–1 of life. This was statistically significant for pneumococcal serotype 9V (GMR 0.67, p-value = 0.04) and 19A (GMR 0.50, p-value = 0.03) (Supplementary Data Table 5). At 13 months of age, there was no apparent difference in GMRs between participants randomised to BCG on day 0–1 and those randomised on day 2–10 (Supplementary Data Table 5).

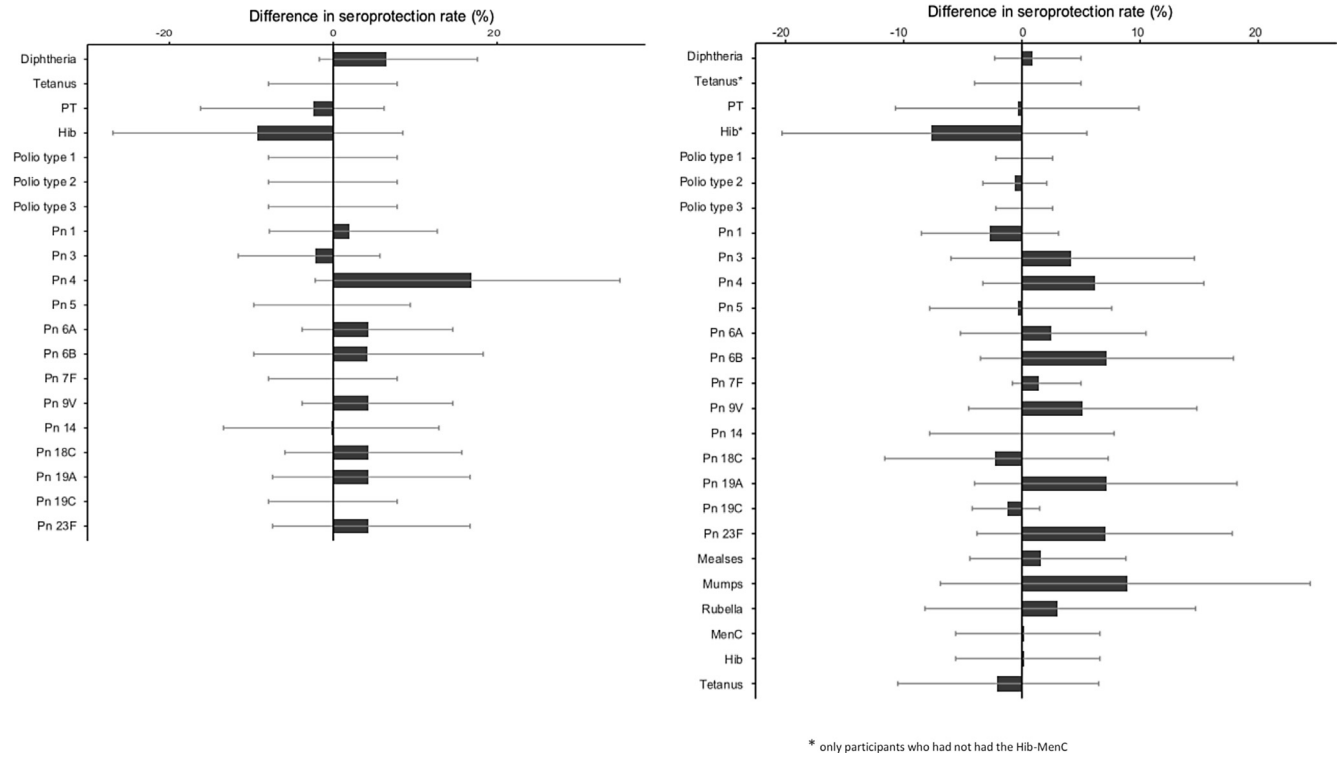

Fig. 2. Differences with 95% CI in seroprotection rates for 26 vaccine antigens in participants at 7 (left panel) and 13 months (right panel) of age. A positive difference indicates a higher seroprotection rate in BCG-vaccinated infants. In right panel, first 20 results relate to responses to primary course of vaccines ending at 6 months of age; last 6 results relate to responses to 12-month vaccines.

Table 3
Geometric mean antibody concentrations (GMCs) and geometric mean antibody ratios (GMRs) in participants at 7 and 13 months of age.

Vaccine antigen	Antibodies to primary course of vaccines ending at 6 months of age measured at 7 months of age						Antibodies to primary course of vaccines ending at 6 months of age measured at 13 months of age					
	BCG-vaccinated (n = 45) GMC (95% CI)	BCG-naïve (n = 46) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value	BCG-vaccinated (n = 169) GMC (95% CI)	BCG-naïve (n = 141) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
Diphtheria[1]	**0.47 (0.38, 0.58)**	**0.34 (0.26, 0.43)**	**1.40 (1.02, 1.92)**	**0.04**	1.18 (0.87, 1.61)[b,c,g]	0.28	0.08 (0.07, 0.10)	0.07 (0.06, 0.08)	1.19 (0.95, 1.50)	0.14	1.16 (0.94, 1.43)[b,g,h,j]	0.17
Tetanus[1]	1.22 (1.01, 1.48)	1.01 (0.81, 1.26)	1.22 (0.91, 1.62)	0.18	1.23 (0.93, 1.64)[e]	0.14	0.48 (0.37, 0.63)[3]	0.39 (0.31, 0.50)[3]	1.24 (0.87, 1.77)[3]	0.24	1.33 (0.95, 1.86)[3,j]	0.10
PT[1]	77.68 (59.87, 100.80)	63.36 (51.12, 78.54)	1.23 (0.88, 1.71)	0.23	1.06 (0.78, 1.45)[b]	0.70	17.81 (15.24, 20.81)	17.13 (14.45, 20.30)	1.04 (0.83, 1.31)	0.74	1.03 (0.83, 1.27)[b,g,h,i,j]	0.80
FHA[1]	64.25 (50.50, 81.73)	63.23 (49.11, 81.42)	1.02 (0.72, 1.43)	0.93	0.91 (0.66, 1.26)[b,c,e]	0.58	19.77 (16.87, 23.17)	18.31 (15.82, 21.18)	1.08 (0.87, 1.34)	0.49	1.09 (0.88, 1.34)[b,e,g,h]	0.43
PRN[1]	66.20 (51.24, 85.53)	55.38 (40.44, 75.82)	1.20 (0.80, 1.78)	0.38	1.05 (0.71, 1.56)[b,c,f]	0.79	12.54 (10.41, 15.11)	11.53 (9.42, 14.11)	1.09 (0.83, 1.43)	0.55	1.08 (0.83, 1.41)[b,e,h]	0.54
Hib[2]	0.54 (0.32, 0.93)	0.48 (0.32, 0.72)	1.13 (0.58, 2.17)	0.72	0.92 (0.48, 1.75)[c,e,g]	0.80	1.05 (0.65, 1,69)[3]	1.22 (0.72, 2.05)[3]	0.86 (0.42, 1.75)[3]	0.68	1.06 (0.54, 2.08)[3,c,h]	0.86
Polio type 1[1]	36.40 (27.76, 47.75)	32.94 (23.23, 46.70)	1.11 (0.71, 1.71)	0.65	1.06 (0.69, 1.63)[c,f]	0.80	10.47 (8.82, 12.42)	11.98 (9.92, 14.47)	0.87 (0.68, 1.13)	0.30	0.86 (0.67, 1.10)[b,h,j]	0.22
Polio type 2[1]	65.26 (49.67, 85.75)	60.83 (42.60, 86.86)	1.07 (0.69, 1.67)	0.75	1.01 (0.65, 1.56)[b,h]	0.97	19.72 (16.21, 24.00)	23.95 (19.51, 29.40)	0.82 (0.62, 1.09)	0.18	0.82 (0.62, 1.09)[b,h]	0.17
Polio type 3[1]	20.20 (14.50, 28.14)	22.72 (16.22, 31.81)	0.89 (0.56, 1.42)	0.62	0.94 (0.60, 1.49)[h]	0.80	8.68 (7.09, 10.62)	9.18 (7.41, 11.36)	0.95 (0.71, 1.27)	0.71	0.92 (0.69, 1.23)[b,e,h,j]	0.57
Pn 1[2]	5.30 (4.16, 6.74)	3.62 (2.55, 5.12)	1.47 (0.96, 2.23)	0.07	1.18 (0.79, 1.75)[a,g,h]	0.42	1.23 (1.07, 1.42)	1.10 (0.96, 1.26)	1.12 (0.92, 1.36)	0.27	1.08 (0.89, 1.30)[b,d,h]	0.44
Pn 3[2]	1.47 (1.21, 1.79)	1.40 (1.14, 1.72)	1.05 (0.79, 1.40)	0.71	1.05 (0.79, 1.40)	0.71	0.57 (0.50, 0.65)	0.51 (0.45, 0.58)	1.12 (0.93, 1.34)	0.23	1.07 (0.90, 1.28)[h]	0.43
Pn 4[2]	0.55 (0.44, 0.69)	0.48 (0.37, 0.63)	1.14 (0.80, 1.62)	0.45	1.02 (0.73, 1.44)[g]	0.89	0.23 (0.21, 0.26)	0.20 (0.18, 0.22)	1.14 (0.98, 1.33)	0.08	1.11 (0.96, 1.29)[b,c,h,j]	0.16
Pn 5[2]	**3.90 (3.07, 4.95)**	**2.57 (1.92, 3.44)**	**1.52 (1.05, 2.20)**	**0.03**	1.28 (0.89, 1.83)[a,g,h]	0.18	0.98 (0.85, 1.13)	0.85 (0.74, 0.99)	1.15 (0.93, 1.41)	0.19	1.11 (0.91, 1.36)[b,g,h,j]	0.30
Pn 6A[2]	5.69 (4.65, 6.97)	4.09 (3.04, 5.48)	1.39 (0.98, 1.98)	0.07	1.21 (0.85, 1.72)[b,g]	0.29	1.07 (0.92, 1.25)	0.93 (0.78, 1.09)	1.16 (0.92, 1.45)	0.21	1.10 (0.90, 1.37)[b,g,h]	0.35
Pn 6B[2]	2.39 (1.55, 3.68)	1.54 (0.97, 2.46)	1.55 (0.83, 2.89)	0.17	1.23 (0.66, 2.31)[d,g]	0.51	0.56 (0.47, 0.67)	0.47 (0.39, 0.57)	1.19 (0.92, 1.55)	0.19	1.16 (0.90, 1.49)[b,h]	0.26
Pn 7F[2]	6.64 (5.35, 8.25)	5.67 (4.44, 7.24)	1.17 (0.85, 1.62)	0.33	1.04 (0.75, 1.44)[b,g]	0.81	1.97 (1.75, 2.22)	1.76 (1.55, 2.01)	1.12 (0.94, 1.33)	0.20	1.08 (0.92, 1.28)[b,g,h]	0.34
Pn 9V[2]	**3.00 (2.47, 3.64)**	**2.03 (1.52, 2.70)**	**1.48 (1.05, 2.09)**	**0.03**	1.25 (0.90, 1.75)[a,g,h]	0.18	0.62 (0.55, 0.71)	0.57 (0.49, 0.66)	1.10 (0.90, 1.33)	0.35	1.04 (0.86, 1.25)[g,h,j]	0.69
Pn 14[2]	2.87 (1.92, 4.29)	2.48 (1.76, 3.51)	1.16 (0.69, 1.95)	0.58	1.16 (0.69, 1.95)	0.58	1.09 (0.93, 1.28)	1.00 (0.85, 1.17)	1.09 (0.87, 1.37)	0.44	1.04 (0.83, 1.30)[g,h,j]	0.74
Pn 18C[2]	3.11 (2.39, 4.05)	2.78 (2.07, 3.75)	1.12 (0.75, 1.65)	0.58	1.01 (0.69, 1.50)[g]	0.94	0.67 (0.59, 0.77)	0.62 (0.55, 0.71)	1.08 (0.89, 1.30)	0.43	1.04 (0.87, 1.24)[b,g,i,j]	0.70
Pn 19A[2]	2.00 (1.47, 2.70)	1.49 (1.15, 1.92)	1.34 (0.91, 1.99)	0.14	1.25 (0.84, 1.85)[b,c]	0.27	0.49 (0.40, 0.61)	0.43 (0.34, 0.53)	1.16 (0.86, 1.56)	0.32	1.07 (0.81, 1.42)[h,j]	0.64
Pn 19F[2]	13.37 (10.29, 17.37)	9.97 (7.67, 12.97)	1.34 (0.93, 1.93)	0.12	1.15 (0.80, 1.66)[b,g]	0.44	3.24 (2.73, 3.83)	2.93 (2.47, 3.47)	1.10 (0.87, 1.40)	0.42	1.04 (0.82, 1.31)[g,h,j]	0.75
Pn 23F[2]	2.96 (2.19, 4.00)	1.86 (1.29, 2.66)	1.59 (1.00, 2.54)	0.05	1.43 (0.90, 2.28)[g]	0.13	0.62 (0.51, 0.74)	0.51 (0.42, 0.62)	1.21 (0.92, 1.59)	0.17	1.15 (0.87, 1.50)[b,e,f,h]	0.32

Vaccine antigen	Antibodies to 12-month vaccines measured at 13 months of age					
	BCG-vaccinated (n = 78) GMC (95% CI)	BCG-naïve (n = 69) GMC (95% CI)	Unadjusted GMR (95% CI)	p-value	Adjusted GMR (95% CI)	p-value
Measles[1]	3.34 (2.75, 4.06)	2.73 (2.01, 3.72)	1.22 (0.86, 1.74)	0.26	1.20 (0.84, 1.70)[e]	0.31
Mumps[1]	67.50 (50.13, 90.88)	52.81 (38.48, 72.49)	1.28 (0.83, 1.97)	0.26	1.14 (0.80, 1.62)[g,h]	0.47
Rubella[1]	54.23 (40.61, 72.42)	52.56 (36.57, 75.53)	1.03 (0.65, 1.63)	0.89	0.90 (0.63, 1.27)[g,h]	0.54
MenC[2]	16.60 (13.43, 20.53)	17.81 (13.76, 23.05)	0.93 (0.67, 1.29)	0.67	0.94 (0.68, 1.30)[e,h]	0.70
Hib[2]	13.71 (9.47, 19.85)	18.39 (12.98, 26.06)	0.75 (0.45, 1.24)	0.26	0.72 (0.44, 1.19)[c,g,h]	0.20
Tetanus[1]	0.95 (0.72, 1.25)	1.07 (0.81, 1.41)	0.89 (0.60, 1.31)	0.55	0.89 (0.60, 1.31)	0.55

BCG = Bacille Calmette-Guérin vaccine, CI = confidence interval, FHA = filamentous haemagglutinin, GMC = geometric mean antibody concentration, GMR = geometric mean antibody ratio, Hib = *H. influenzae* type b, MenC = meningococcus C, Pn = pneumococcus serotype, PT = pertussis toxin, PRN = pertactin.

[1] IU/ml.
[2] μg/ml.
[3] Only participants who have not had Hib-MenC.
[a] Maternal BCG status.
[b] Maternal dTpa during pregnancy.
[c] Gestational age.
[d] Delivery mode.
[e] Sex.
[f] Birth weight.
[g] Age at bleeding.
[h] Time between immunisation and bleeding.
[i] Age at randomisation.
[j] MMR/Hib-MenC immunisation status.

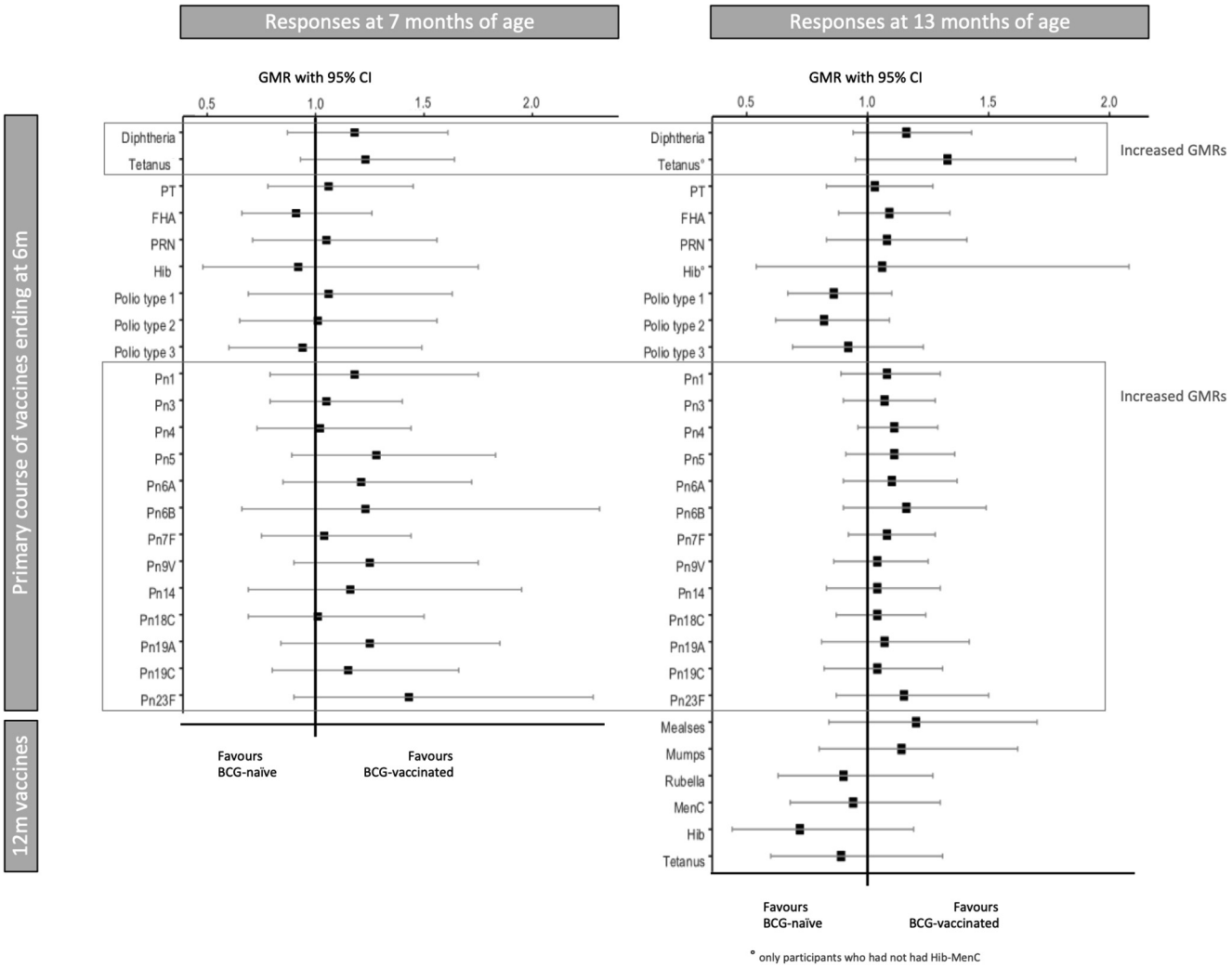

Fig. 3. GMRs with 95% CI for 26 vaccine antigens in participants at 7 and 13 months of age.

4. Discussion

BCG immunisation has heterologous effects on the infant immune system [37]. It is a low-cost and readily available intervention with a well-established safety profile. To date, only a few studies have investigated the effect of previous or concurrent administration of BCG on heterologous vaccines responses [21]. A number of these studies have reported that BCG immunisation is associated with increased vaccine responses to HepB, polio, pneumococcus and influenza [23–26]. However, a recent study in Denmark found higher GMCs against pertussis and pneumococcus (statistically significant for serotypes 9V, 18C and 19F) only when BCG was given after the first day of life [27].

Our study includes the broadest range of vaccine responses tested to date for BCG immunomodulatory effects and is the first to include responses to meningococcal and MMR immunisation, as well as to three different pertussis and polio antigens. Our results suggest that neonatal BCG immunisation might lead to higher GMCs and seroprotection rates for diphtheria, tetanus and pneumococcal antigens, and a reduced response and seroprotection rate for Hib. Although our findings only reached statistical significance for a few of the pneumococcal serotypes (9V, 19F and 23F) when BCG was given after the first day of life, the consistent direction of change in GMRs at both 7 and 13 months of age suggest a possible effect of BCG on antibody responses. Our results are consistent with those from a recent trial in Denmark [27] which reported a stronger effect on vaccine responses when BCG vaccine was administered after the first day of life.

Although the observed differences in GMCs measured approximately one month after immunisation were relatively small, these differences in antibody response might influence the duration of protection. This is highlighted by the differences in the proportion of participants who had antibody concentrations above the protective threshold at 13 months of age, although the clinical significance of these differences is uncertain.

The precise mechanisms underlying the immunomodulatory effects of BCG immunisation are the subject of ongoing investigation. Studies show that BCG immunisation affects both innate [24,38,39] and T cell immunity [40]. Epigenetic reprogramming by BCG alters innate immune cells that my function as antigen-presenting cells [41,42]. Changes in innate immune function early in life, together with increased expression of co-stimulatory molecules or cytokine responses, might mechanisms by which BCG influences adaptive immune responses to other vaccines [38,43].

Although the effects of BCG on T-cell immunity are well established [5,24,40], few studies have investigated the influence of BCG on cellular responses to heterologous vaccines. A case-control study showed that BCG given at birth leads to an increase in interferon (IFN)-gamma-producing spot-forming cells (SFC) to tetanus and polio, but not to HepB antigens [44], and a randomised trial

of BCG-Tice given at birth led to an increase in IFN-gamma (as well as interleukin (IL)-5- and IL-13-) producing T cells to HepB antigens [24]. Augmented cellular responses induced by BCG is therefore another possible mechanism by which BCG influences vaccine responses, especially in infants, who have reduced T-helper 1 cell responses to many pathogens and toxins [45]. The possibility that BCG only has indirect effects on B-cells through innate immunity and T-cells [24,44] might be one explanation for the small magnitude of the differences in heterologous vaccine responses between BCG-vaccinated and BCG-naïve infants.

Different BCG vaccine strains are associated with differences in vaccine efficacy. BCG-Japan has been suggested to have superior efficacy compared to BCG-Russia in protecting against TB [46]. TB-specific responses (mycobacterial-specific polyfunctional and cytotoxic T cells) are higher after immunisation with BCG-Denmark and BCG-Japan compared with BCG-Russia [47]. BCG-Denmark also leads to higher *in vitro* cytokine responses to both specific and non-specific stimuli compared with BCG-Russia and BCG-Bulgaria [47,48]. It is therefore possible that different BCG vaccine strains also have dissimilar heterologous effects. To date, most studies investigating the effect of BCG on heterologous vaccine responses, including the recent study from Denmark [27] and ours, have used BCG-Denmark, which is a strain that has been associated with superior efficacy for both specific and non-specific effects [49].

The strengths of our study are the use of samples from a randomised trial, the detailed participant demographic and clinical data allowing for adjustment for possible confounding factors, the homogeneity in the vaccines given and the range of measured antibody responses. The limitations of the study, aside from the small sample size and consequent risk of type-2 error, include the fact that participants were not specifically randomised for the outcome of this study, that not all participants had antibodies measured exactly 4 weeks after immunisation, and that antibody concentrations were not measured pre vaccination.

The results of this study add to the evidence that BCG immunisation at birth modulates the early-life immune system, including the humoral response to heterologous vaccines. The immunomodulatory effects of BCG might be used to improve vaccine efficacy and duration of protection on a population level. They also need to be taken into account when considering replacing BCG with new vaccines, as well as when deciding on recommendations for discontinuation of universal BCG immunisation programmes [3]. Future studies investigating the immunomodulatory effects of BCG should focus on the differences between BCG vaccine strains and on the optimal timing of administration.

5. Competing interests

The authors declare that they have no competing interests.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.03.016.

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