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Influence of *Mycobacterium bovis* Bacillus Calmette-Guérin on Antibody and Cytokine Responses to Human Neonatal Vaccination[1]

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The immaturity of the immune system increases the susceptibility of young infants to infectious diseases and prevents the induction of protective immune responses by vaccines. We previously reported that *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccination induces a potent Th1 response to mycobacterial Ags in newborns. In this study, we evaluated the influence of BCG on the response to unrelated vaccines given in early life. Newborns were randomly allocated to one of three study groups receiving BCG at birth, when infants received their first dose of hepatitis B and oral polio vaccines; at 2 mo of age, when infants received their first dose of diphtheria and tetanus vaccines; or at 4.5 mo of age, when immune responses to vaccines were measured. Administration of BCG at the time of priming markedly increased the cellular and Ab responses to the hepatitis B vaccine, but had only a limited influence on the cytokine response to tetanus toxoid and no effect on the Ab responses to tetanus and diphtheria toxoids. Although BCG induced a potent Th1-type response to mycobacterial Ags, it promoted the production of both Th1- and Th2-type cytokines in response to unrelated vaccines. The effect of BCG was apparent at the systemic level, as it increased the Ab response to oral polio vaccine. These results demonstrate that BCG influences the immune response to unrelated Ags in early life, likely through its influence on the maturation of dendritic cells. ***The Journal of Immunology*****, 2002, 168: 919–925.**

Infectious diseases are the main cause of mortality in young children, causing the death of ~4 million infants yearly (1, 2). This increased susceptibility to infections is related to an immaturity of the immune system that also prevents the induction of protective immune responses by vaccines (1, 2). Studies in mice indicate that immune responses at birth are often biased toward the Th2 type and defective in the Th1 type, the central defense mechanism against intracellular pathogens (1, 3). Relatively little is known about helper T cell responses in human newborns. Recent data showing that human cord blood-derived dendritic cells have a profound defect in the production of IL-12, a cytokine playing a central role in the differentiation of Th1 lymphocytes, suggest that type 1 responses could also be defective in human newborns (4). In contrast, we observed that *Mycobacterium bovis* bacillus Calmette-Guérin (BCG)[3] vaccination induces a potent Th1-type immune response at birth in humans as in mice (5–7). This could be related to the potent APC-activating properties of BCG and/or to its persistence during the maturation of the immune system (1). By inducing a potent Th1-type response at birth, BCG could influence the immune response to unrelated Ags. We have observed that BCG immunization during the first week of life is associated with a reduced risk of atopy in children in Guinea-Bissau (8). BCG is the world's most widely used vaccine and although its efficacy against adult disease is variable, it protects against childhood tuberculosis (9). Therefore, an influence of BCG on immune responses to unrelated Ags would have important public health implications.

The objective of this study was to evaluate whether the Th1-type immune response induced by BCG could influence the T cell and the Ab responses to unrelated vaccine Ags. Children were recruited at birth and were randomly allocated to one of three study groups: those receiving BCG at birth, at 2 mo, or at 4.5 mo of age. This design allowed us to analyze the effect of BCG when given (1) at the time of priming with hepatitis B vaccine (HBV) and oral polio vaccine (OPV) at birth or with diphtheria/pertussis/tetanus vaccine (DPT) at 2 mo of age or (2) at the time of booster immunization with HBV and OPV at 2 mo of age. Immune responses to vaccines were measured at 4.5 mo of age and, therefore, were not influenced by BCG in control infants who received BCG at 4.5 mo.

Materials and Methods

Study design and population

This study was a prospective and randomized trial approved by The Gambia Government/Medical Research Council Ethics Committee. Newborns were enrolled at birth at Royal Victoria Hospital (Banjul, The Gambia) after maternal informed consent. The following were excluded from the study: neonates born to mothers with systemic infection at the time of

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[3] Abbreviations used in this paper: BCG, *Mycobacterium bovis* bacillus Calmette-Guérin; HBV, hepatitis B vaccine; OPV, oral polio vaccine; DPT, diphtheria-pertussis-tetanus toxoids vaccine; PPD, purified protein derivative; HbsAg, hepatitis B surface Ag; TT, tetanus toxoid; DT, diphtheria toxoid; DC, dendritic cell; CI, confidence interval.

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Table I. *Vaccination and sampling schedule*[a]

Vaccine	Age (mo)					
	0	1	2	3	4	4.5
BCG group 1	X					
BCG group 2			X			
BCG controls						X
HBV (groups 1, 2, controls)	X		X		X	
OPV (groups 1, 2, controls)	X	X	X	X		
DTP (groups 1, 2, controls)			X	X	X	
Sampling (groups 1, 2, controls)	X		X			X

[a] All vaccines, except OPV, were given in the left deltoid area.

delivery, newborns with congenital defects, newborns with birth weight less than 2.5 kg, and twins. When a person with a suggestive history of tuberculosis was found in the compound, the newborn was vaccinated with BCG and was excluded from the study. Enrolled newborns were randomly allocated in blocks of six to one of three groups: those receiving BCG immediately (group 1), those receiving BCG at 2 mo of age (group 2), or those receiving BCG at 4.5 mo of age (control infants; Table I). All other vaccines were given according to recommendation of the Gambian Expanded Program for Immunization, including OPV at birth, and at 1, 2, and 3 mo; HBV at birth, and at 2, and 4 mo; and DPT at 2, 3, and 4 mo (Table I). Blood samples were collected at birth (cord blood), and at 2, and 4.5 mo of age (Table I). One hundred fifty-one newborns were enrolled into the study. One hundred four were studied at 2 mo, including 35, 35, and 34 infants vaccinated with BCG at birth, and at 2, or 4.5 mo of age, respectively. Eighty-five were studied at 4.5 mo, including 28, 29, and 28 infants vaccinated with BCG at birth, and at 2 or 4.5 mo of age, respectively. Infants who were lost to follow-up had similar socio-demographic and clinical characteristics at birth as that of followed-up infants. Prevaccination T cell responses to BCG and HBV were measured in a consecutive series of 23 cord blood samples.

Vaccines

BCG (0.05 ml; Aventis Pasteur, Lyon, France) was given intradermally in the left arm. HBV (0.5 ml, Engerix B; Glaxo SmithKline, Rixensart, Belgium) and whole-cell DPT (0.5 ml; Aventis Pasteur) were injected i.m. in the left arm. OPV (Sabin; Glaxo SmithKline) was given orally.

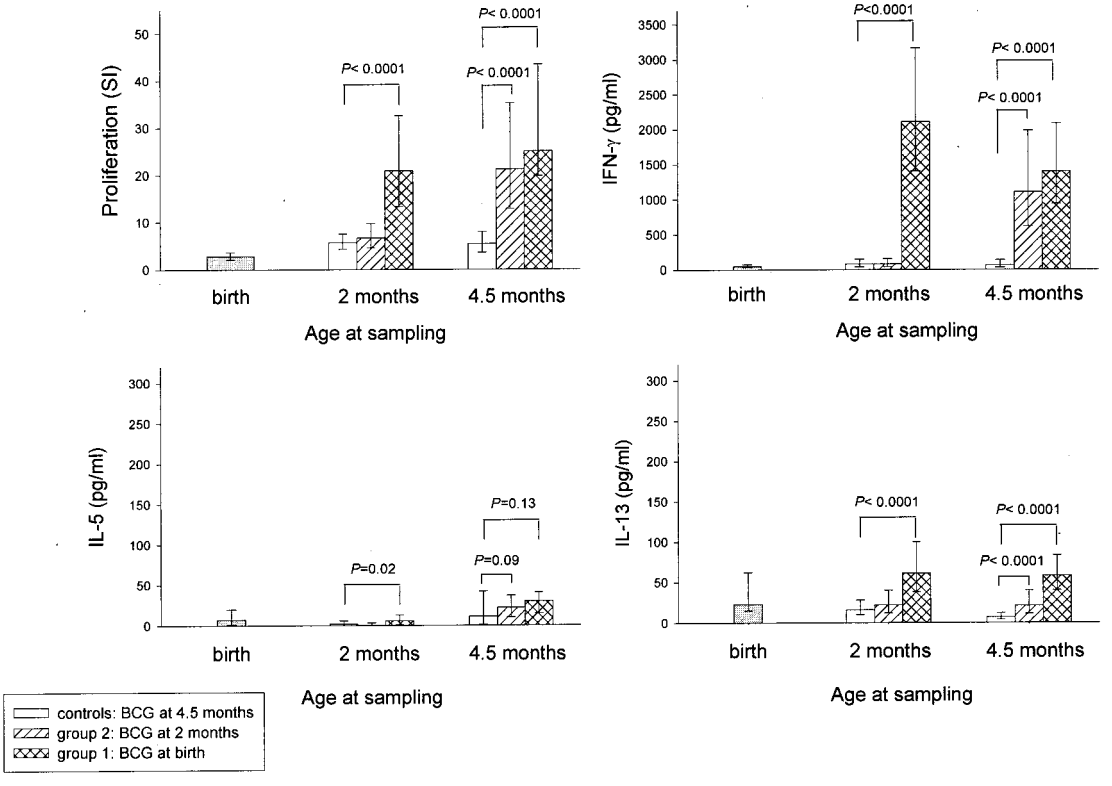

FIGURE 1. Lymphocyte response to BCG. Immune response to BCG was assessed by measuring cell proliferation as well as IFN-γ, IL-5, and IL-13 production in response to PPD in vitro restimulation. Responses to PPD were measured at birth in a series of cord blood (▨, $n = 23$); at 2 mo of age, postvaccination in infants who received BCG at birth (▩, group 1, n infants tested ranged from 30 to 32 depending on the response measured), and prevaccination in infants scheduled to receive BCG at 2 mo (▨, group 2, $n = 26–34$) or 4.5 mo of age (□, control infants, $n = 27–32$), at 4.5 mo of age, postvaccination in infants who received BCG at birth (▩, $n = 23–27$) or at 2 mo of age (▨, $n = 23–29$), and prevaccination in infants scheduled to receive BCG at 4.5 mo (□, $n = 22–26$). Data are expressed as geometric means and 95% confidence interval (CI) except IL-5 concentrations that are expressed as median and 95% CI. The figure shows the test p value obtained in a regression model adjusting for birth weight, ethnicity, date and season of delivery, sex, and birth order. SI, stimulation index.

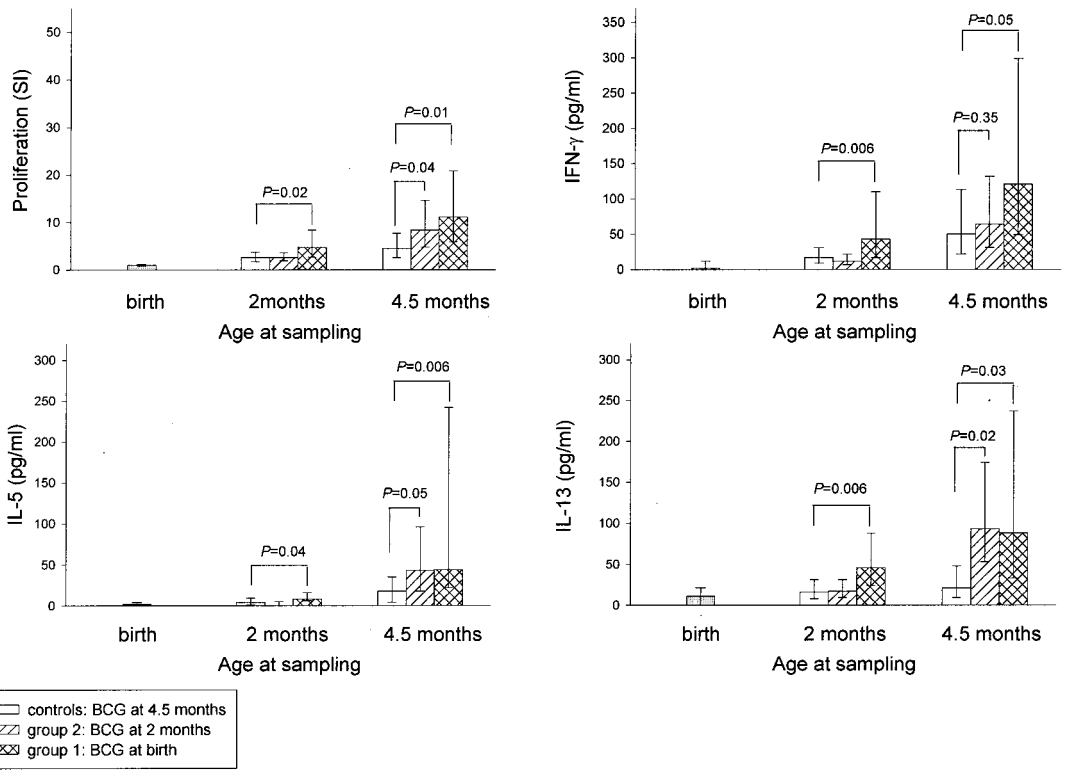

FIGURE 2. Lymphocyte response to HBV. All infants were vaccinated with HBV at birth and at 2 and 4 mo of age. Immune response to HBV was assessed by measuring cell proliferation as well as IFN-γ, IL-5, and IL-13 production in response to HBs Ag in vitro restimulation. Responses to HBs Ag at birth were measured in a series of cord blood (▨, $n = 23$). Responses to HBs Ag were measured at 2 and 4.5 mo in infants who received BCG at 4.5 mo of age (□, control infants, n tested ranged from 27 to 32 at 2 mo and from 22 to 26 at 4.5 mo, depending on the response measured), at 2 mo of age (▨, group 2, $n = 26–34$ at 2 mo and $n = 23–29$ at 4.5 mo), or at birth (▩, group 1, $n = 30–32$ at 2 mo and $n = 23–27$ at 4.5 mo). Data are expressed as geometric means and 95% CI except IL-5 concentrations that are expressed as median and 95% CI. The figure shows the test p value obtained in a regression model adjusting for birth weight, ethnicity, date and season of delivery, sex, and birth order. SI, stimulation index.

In vitro lymphocyte responses to vaccine Ags

PBMC were isolated by density gradient centrifugation (Lymphoprep; Nycomed, Oslo, Norway) and were resuspended in complete RPMI 1640 medium supplemented with 10% human AB serum (Sigma-Aldrich, St. Louis, MO). PBMC (2.10^5/200 μl) were incubated with Ags including purified protein derivative (PPD, RT49, 10 μg/ml; Statens Serum Institut, Copenhagen, Denmark), hepatitis B surface Ag (HbsAg, 2 μg/ml; Glaxo SmithKline) and tetanus toxoid (TT, 2 μg/ml; Chiron Behring, Marburg, Germany), PHA (PHA-L, 10 μg/ml; Sigma Chemicals, Poole, Dorset, U.K.), or medium alone. Methyl-[^{3}H]thymidine (1 μCi/well, Amersham Life Science, Buckinghamshire, U.K.) was added for the final 17 h of culture to assess cell proliferation. Thymidine incorporation was measured by liquid scintillation using a Betaplate reader (LKB1205; LKB Instruments, Turku, Finland).

Cytokine assays

Cytokine concentrations were measured in supernatants collected on day 2 (PHA) or day 6 (medium and Ags) using commercially available reagents (IFN-γ and IL-5; BioSource Europe, Fleurus, Belgium; IL-13; Diaclone, Besançon, France).

Ab assays

Neutralizing Abs to poliovirus type 1 were measured as recommended by the World Health Organization (23). Dilutions of heat-inactivated sera were incubated with poliovirus (100 tissue culture infective $dose_{50}$) for 3 h. The mixture was incubated with Hep-2 cells for 5 days. The serum Ab titer was taken as the highest serum dilution protecting 50% of cultures against virus challenge and was converted into international units. Ab concentrations to TT, DT, and HBsAg were determined by ELISA on plates coated with TT (Chiron Behring), DT (Aventis Pasteur), or HBsAg (Glaxo SmithKline). Incubation of serum samples was followed by successive addition of biotinylated goat anti-human IgG (Sigma Chemicals) and extravidine-peroxidase (for TT and DT Abs) or peroxidase-coupled goat anti-human IgG (Cappell-ICN, Costa Mesa, CA, for HBsAg Abs) and ABTS substrate. Ab concentrations were calculated with the Softmax PRO software (Molecular Devices, Sunnyvale, CA) by comparison with standard curves (4-parameter fitting) using international standards of reference. Values below the assay cutoff (i.e., <100 mIU/ml (tetanus, diphtheria) or 150 mIU/ml (hepatitis B)) were arbitrarily given a value of one-half the cut-off for determination of geometric mean titers.

Statistical analysis

Background production of cytokines measured in control wells was subtracted from that measured in Ag-stimulated wells. After logarithm transformation, data were compared using the t test. Where there were either many nonresponders or persistent skewing of data after log transformation, the Wilcoxon test was used. Multiple regression analysis was conducted to allow for potential confounders. Despite randomization, group 2 infants had a lower birth weight ($p = 0.02$). Thus, we adjusted for birth weight, ethnicity, date and season of delivery, sex, and parity of mother. Those measurements with many nonresponders were categorized and analyzed by ordinal logistic regression. Tables and figures show adjusted p values for three pairwise comparisons. At 2-mo sampling, groups vaccinated with BCG at 2 or 4.5 mo were combined and compared with the group vaccinated at birth. At 4.5-mo sampling, the group vaccinated with BCG at 4.5 mo was compared with groups vaccinated at birth or 2 mo, separately. Statistical significance was assessed at the two-sided 0.05 level. All statistical analysis was done using Stata software (version 6; Stata, College Station, TX).

Results

BCG induces a Th1-type immune response at birth and at 2 mo of age

We first confirmed that BCG induced a Th1-type immune response in our study population (6, 7). Infants vaccinated at birth with BCG

(group 1) produced high concentrations of IFN-γ and showed strong proliferative responses to PPD at 2 and 4.5 mo of age (Fig. 1). In contrast, only low production of type 2 cytokines, IL-5 and IL-13, was detected. Similar responses were observed at 4.5 mo in infants who had been vaccinated at 2 mo of age (group 2), whereas only minimal responses were detected in control infants who had not received BCG.

Influence of BCG on the cytokine response to HBV

In control infants who had not received BCG, HBV immunization at birth, and at 2 and 4 mo of age induced the production of both type 1 and type 2 cytokines in response to HBsAg (Fig. 2). Administration of BCG at the time of priming markedly increased the lymphocyte response to HBV. Cytokine and proliferative responses to HBsAg were significantly higher in infants vaccinated at birth (group 1) than in infants who had not received BCG. The production of type 1, IFN-γ, and type 2 cytokines, IL-5 and IL-13, were enhanced by BCG. This effect was already apparent at 2 mo of age, after a single dose of HBV, and was further enhanced at 4.5 mo of age. Infants vaccinated with BCG at 2 mo of age (group 2), at the same time as the second dose of HBV, produced significantly higher concentrations of IL-5 and IL-13 and had stronger proliferative responses to HBsAg than infants who had not received BCG. These responses were similar to those measured in infants who received BCG at the time of priming. In contrast, BCG vaccination at 2 mo did not significantly influence IFN-γ response to HBV. The specificity of these differences for vaccine Ags was confirmed by the lack of influence of BCG on responses to PHA in the three groups (data not shown).

Influence of BCG on the Ab responses to HBV and OPV

As shown in Fig. 3, the increased lymphocyte response to HBV was associated with an increased Ab response. Infants vaccinated at birth with BCG (group 1) had significantly higher anti-HBs IgG than control infants who had not received BCG. This effect was apparent at 2 mo of age and was maximal after immunization with three doses of HBV. In contrast, administration of BCG at the same time as the second dose of HBV (group 2) did not influence the Ab response to HBV. Fig. 4 shows individual data obtained from three representative infants vaccinated at birth with BCG (group 1) and three representative controls. In most infants vaccinated at birth with BCG, the increased Ab response to HBV was associated with an increased production of both type 1, IFN-γ, and type 2 cytokines, IL-5 and IL-13, in response to HBsAg. The effect of BCG on proliferative responses to HBsAg was more variable.

BCG vaccination also enhanced the Ab response to OPV, but only when given at the time of boosting. OPV vaccination at birth, and at 1, 2, and 3 mo of age stimulated the production of high concentrations of anti-poliovirus type 1-neutralizing Abs (Fig. 3). A marked enhancement of the Ab response to OPV was observed in infants vaccinated with BCG at 2 mo of age (group 2), at the same time as the third dose of OPV. In contrast, administration of BCG at the time of OPV priming (group 1) did not significantly increase the Ab concentration to poliovirus type 1.

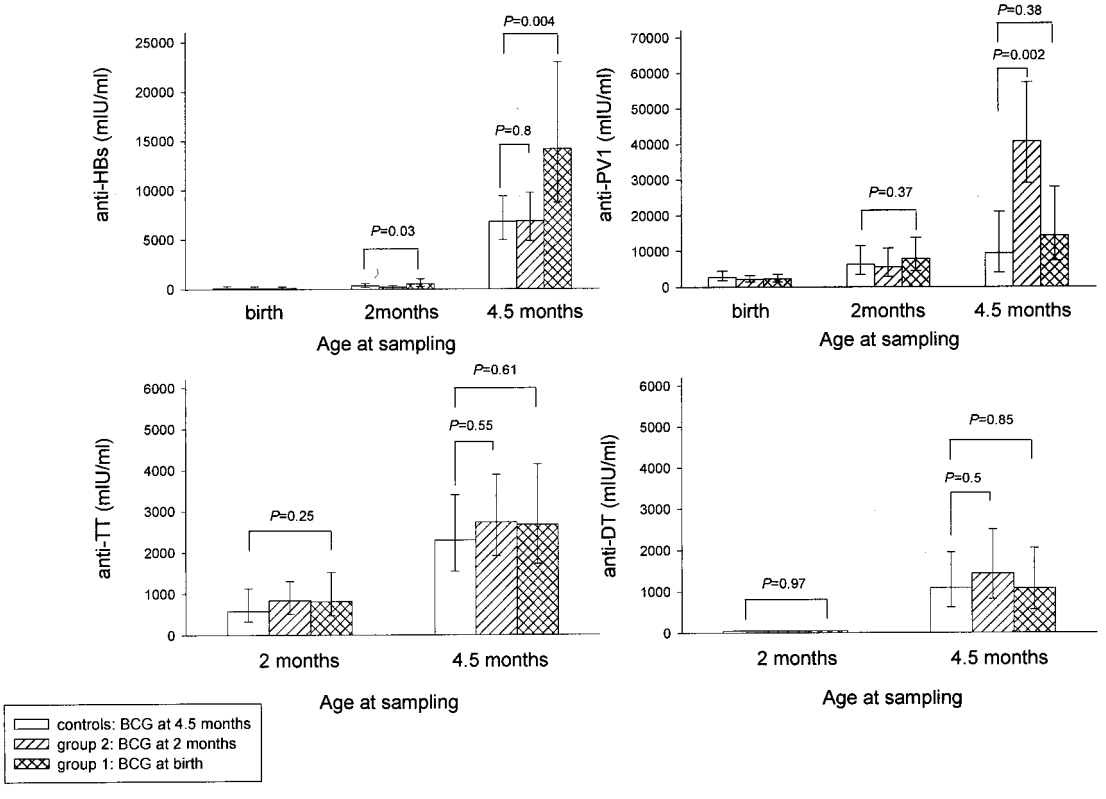

FIGURE 3. Antibody response to HBV, OPV, TT, and DT. All infants were vaccinated with HBV, OPV, and DPT as described in Table I. Anti-HBs and OPV-1 Ab concentrations (*upper panels*) were measured at birth, and at 2 and 4.5 mo of age in infants vaccinated with BCG at 4.5 mo of age (□, control infants, *n* samples tested at birth = 44, at 2 mo = 34, and at 4.5 mo = 28), at 2 mo of age (▨, group 2, *n* samples tested at birth = 44, at 2 mo = 34, and at 4.5 mo = 29), or at birth (▩, group 1, *n* samples tested at birth = 45, at 2 mo = 34, and at 4.5 mo = 27). Anti-TT and DT Ab concentrations (*lower panels*) were measured at 2 and 4.5 mo of age in infants vaccinated with BCG at 4.5 mo of age (□, *n* samples tested at 2 mo = 35 and at 4.5 mo = 28), at 2 mo of age (▨, *n* samples tested at 2 mo = 35 and at 4.5 mo = 29), or at birth (▩, *n* samples at 2 mo = 35 and at 4.5 mo = 28). HBs, TT, and DT Ab results are expressed as median mIU/ml (95% CI), and OPV Ab results are expressed as geometric means mIU/ml (95% CI). The figure shows the test *p* value obtained in a regression model adjusting for birth weight, ethnicity, date and season of delivery, sex, and birth order.

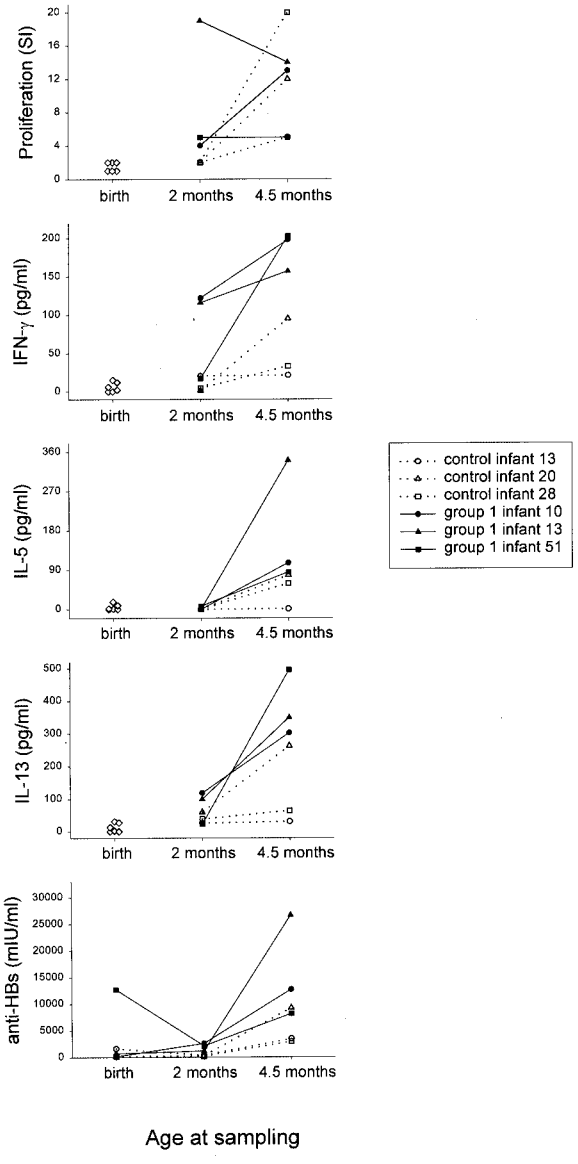

FIGURE 4. Individual responses to HBV. All infants were vaccinated with HBV at birth and at 2 and 4 mo of age. Immune response to HBV was assessed by measuring proliferative, IFN-γ, IL-5, and IL-13 responses to in vitro restimulation with HBsAg as well as serum concentrations of anti-HBs Abs. The figure shows individual responses measured in three representative infants vaccinated at birth with BCG (group 1, ●, ▲, and ■) and three representative infants vaccinated at 4.5 mo of age (controls, ○, △, and □). Proliferative and cytokine responses were measured at birth in a consecutive series of 23 newborns; data from 6 representative newborns are shown. SI, stimulation index.

Influence of BCG on the cytokine response to TT

DPT immunization at 2, 3, and 4 mo of age induced the production of both type 1 and type 2 cytokines in response to TT (Fig. 5). BCG vaccination at the time of priming had a limited but significant influence on this response. Infants vaccinated with BCG at 2 mo of age (group 2) showed higher cytokine responses to TT than control infants who had not received BCG, but these differences were only significant for IL-5 and IL-13. BCG vaccination also had a limited but significant influence on the cytokine response to TT when given at birth, 2 mo before the first dose of DPT. Infants vaccinated at birth with BCG (group 1) showed significantly higher IL-13 responses to TT than control infants who had not received BCG (Fig. 5). These IL-13 responses to TT were similar to those measured in infants who received BCG at 2 mo of age. In contrast, no significant effect of BCG vaccination at birth was observed on proliferative, IFN-γ, or IL-5 responses to TT.

Influence of BCG on the Ab responses to TT and DT

Relatively high concentrations of anti-TT IgG were detected at 2 mo of age (Fig. 3) as a result of the maternal immunization practice in The Gambia (10). BCG administration with DPT priming did not influence the Ab response to TT as measured at 4.5 mo. The Ab response to DT was also similar in infants who received BCG at 2 mo (group 2) and in those who had not received BCG (controls). Infants who received BCG at birth (group 1) also had similar Ab responses to TT and DT than those who had not received BCG (controls).

Discussion

This study is the first to demonstrate that BCG vaccination in early life significantly enhances T and B cell responses to unrelated vaccine Ags. Unexpectedly, BCG vaccination affected responses to various vaccines differently whether administered at the time of priming, boosting, or even before priming; it enhanced both Th1 and Th2 cytokine responses to unrelated Ags; and it extended its influence on Ab responses to the mucosally administered oral polio vaccine.

The effect of BCG on unrelated vaccine Ags injected in the same arm was found to depend on the vaccine Ag and on the timing of immunization in relation to BCG administration. The strongest influence of BCG was observed on responses to HBV, one of the most immunogenic vaccines administered to infants (1). In infants who had not received BCG, neonatal HBV vaccination induced the production of both type 1 and type 2 cytokines in response to HBsAg. This response was qualitatively similar but of lower magnitude than that measured in adults (M. O. C. Ota, J. Vekemans, S. E. Schlegel-Haueter, K. Fielding, H. Whittle, P. H. Lambert, K. P. W. J. McAdam, C. A. Siegrist, and A. Marchant, manuscript in preparation). The administration of BCG at the time of HBV priming at birth markedly increased the cytokine (IFN-γ, IL-5, and IL-13), proliferative, as well as Ab responses to HBV. When given at 2 mo of age, at the time of booster HBV immunization, BCG also increased the production of type 2 cytokines, but not the IFN-γ, proliferative, or Ab responses. BCG also had a significant although limited influence on the immune response to priming with TT. BCG administration at 2 mo of age, together with the first dose of DPT, increased the production of type 2 cytokines in response to TT, but did not significantly influence TT-specific IFN-γ, proliferative, or Ab responses. No significant effect was observed on the Ab response to DT (for which cellular responses were not assessed), in keeping with data reported by Simondon et al. (11) showing no influence of BCG vaccination on Ab response to pertussis vaccine. A surprising finding was that a similar increase of TT-specific IL-13 responses was observed when BCG was given at birth, 2 mo before DTP priming. Thus, BCG may influence T and B cell responses to unrelated vaccine Ags that are administered at the same injection site either simultaneously or even several weeks later.

One of the central characteristics of Th1 and Th2 cells is that they reciprocally inhibit their differentiation and function (12). Given the induction of a potent Th1-type response to mycobacterial Ags such as PPD, the observation that BCG promoted both type 1 and type 2 cytokine responses to unrelated vaccine Ags was unexpected. In fact, the production of Th2 cytokines was more frequently increased than that of Th1. The mechanism underlying the stimulation of T cell responses by BCG is likely to be related

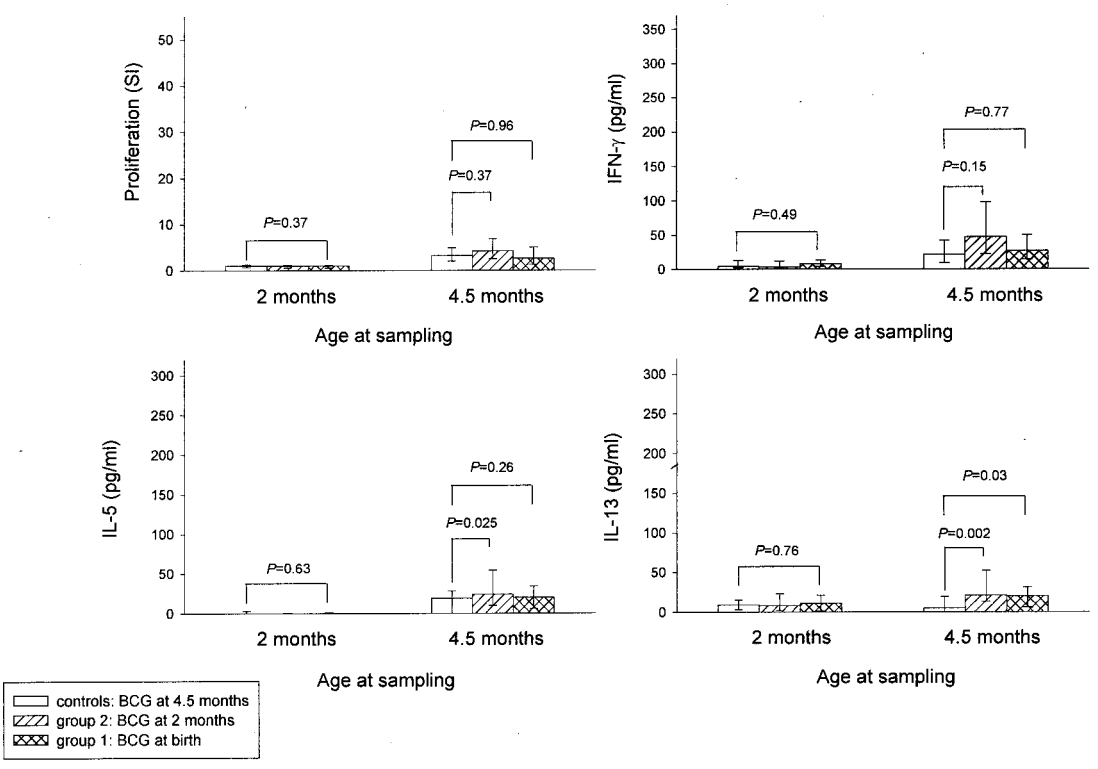

FIGURE 5. Lymphocyte response to TT. All infants were vaccinated with DPT at 2 and 4 mo of age. Immune response to TT was assessed by measuring cell proliferation as well as IFN-γ, IL-5, and IL-13 production in response to TT in vitro restimulation. Responses to TT were measured at 2 and 4.5 mo in infants who received BCG at 4.5 mo of age (□, control infants, *n* tested ranged from 27 to 32 at 2 mo and from 22 to 26 at 4.5 mo, depending on the response measured), at 2 mo of age (▨, group 2, $n = 26–34$ at 2 mo and $n = 23–29$ at 4.5 mo), or at birth (▩, group 1, $n = 30–32$ at 2 mo and $n = 23–27$ at 4.5 mo). Data are expressed as geometric means and 95% CI except all IL-5 and IL-13 concentrations as well as IFN-γ concentrations measured at 2 mo, which are expressed as median and 95% CI. The figure shows the test *p* value obtained in a regression model adjusting for birth weight, ethnicity, date and season of delivery, sex, and birth order. SI, stimulation index.

to its influence on the maturation of neonatal DC, as DC are essential Ag-presenting cells for the priming of naive T cells (13). Mycobacteria, including BCG, activate adult DCs, increasing their production of IL-12 and their expression of costimulatory molecules, which support the induction of Th1 responses (14, 15). However, recent data indicate that DC derived from cord blood monocytes have defective expression of IL-12 (p35) gene as well as of membrane costimulatory molecules (4). Under suboptimal conditions of costimulation, IL-12 was shown to stimulate the production of both type 1 and type 2 cytokines by neonatal CD4 T cells (16). Thus, the promotion of type 1 and type 2 cytokine (or even predominant Th2 cytokine) responses by BCG in early life could be related to a suboptimal state of activation of neonatal DC. Further studies are needed to evaluate whether the promotion of type 2 cytokine responses to unrelated Ags by BCG is a characteristic feature of neonatal responses, or whether it is also observed in adults.

The mechanisms underlying the influence of BCG on the priming of Ab responses to unrelated vaccine Ags is likely to involve enhanced activation of T lymphocytes by APC. In support of this hypothesis, the enhanced HBV Ab response following BCG vaccination at birth paralleled the marked increase in the production of T cell cytokines, whereas the more modest increase in the cytokine response to TT was not associated with significant changes in the TT Ab response. The increased Ab response to HBV priming was marginal when assessed at 2 mo of age, whereas a marked increase was observed at 4.5 mo of age. This indicates that BCG at birth had a limited impact on early life plasmocyte differentiation, but markedly enhanced induction of memory B cells. This is in accordance with studies in mice suggesting that induction of memory B cells occurs much earlier during life than plasmocyte differentiation (1). The enhancement of APC-T cell interactions did not appear to be sufficient to promote the Ab response to booster HBV immunization. Indeed, although a similar increase in the production of type 2 cytokines was observed in infants who received BCG at the time of priming or boosting, the Ab response was not increased by BCG when given at the time of boosting.

BCG considerably influenced the Ab response to mucosally administered OPV. Newborns develop a relatively weak response to OPV (1), and BCG vaccination at birth did not significantly increase OPV Ab responses. In contrast, BCG markedly enhanced OPV Ab response when given at 2 mo of age, together with the third dose of OPV. These data suggest that the maturation of B cell responses to OPV is associated with an increased responsiveness to the influence of BCG. The marked increase in the response to OPV suggests that intradermal BCG vaccination can influence immune responses to unrelated Ags at the systemic level. A systemic modulation of immune responses was recently observed in patients with intestinal helminth infection (17). De Smedt et al. (18) reported that injection of bacterial products induces the migration and the maturation of splenic DC in mice. A similar mechanism could be involved in the up-regulation of systemic immune responses by BCG.

This study has several important implications. First, BCG administration, in its native form or as a live vector, could be a useful strategy in improving the immunogenicity of vaccines in early life (19). Second, BCG vaccination in early life could influence immune responses to unrelated infectious pathogens. A recent report

indicating that BCG vaccination is associated with reduced mortality in infants in Guinea-Bissau emphasizes the potential public health relevance of this hypothesis (20). The possible influence of BCG on the development of allergic reactions remains controversial (8, 21, 22). The enhancement of Th2 responses by BCG observed in this study suggests that the influence of BCG on the immune response to allergens is likely to be more complex than a polarization toward Th1 responses.

Acknowledgments

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