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Population differences in immune responses to BCG in infancy[1]

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Abstract

BCG vaccination induces a marked increase in the IFNγ response to M.tb PPD in UK, but not Malawian adolescents. We hypothesized that PPD-induced IFNγ following BCG vaccination would be similar in infants in the two countries. Infants were BCG-vaccinated in the first 3-13 weeks of life. Three months post-BCG, 100% (51/51) of UK infants made an IFNγ response to M.tb PPD, compared to 53% of Malawian infants in whom responses varied by season of birth.

We conclude that population differences in immune responses following BCG vaccination are observed in infants, as well as in young adults.

Keywords

BCG vaccination; Infant immune responses; IFNγ

Introduction

Bacille Calmette-Guerin (BCG), a live attenuated strain of *Mycobacterium bovis*, is the only vaccine available against tuberculosis and has been part of the WHO Expanded Program on Immunisation (EPI) since 1974. Most countries, including Malawi, give BCG at or soon after birth [1]. A recent policy change in the UK has led to targeted vaccination of high risk infants [2].

Clinical trials of BCG show variable efficacy (0% to 80%) against pulmonary tuberculosis in adults, providing good protection against pulmonary tuberculosis in the UK, but little

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protection in Malawi [3]. In infants, BCG vaccination trials show consistently high efficacy against the severe forms of childhood tuberculosis [4].

We observed a large increase in IFNγ production to *M. tuberculosis* purified protein derivative (M.tb PPD) in UK BCG-vaccinated adolescents, but a relatively small mean change in Malawian adolescents, who made an IFNγ response to mycobacterial antigens prior to vaccination [5]. Differing immune responses to BCG vaccination in the two populations may be due to differing levels of prior exposure to environmental mycobacteria [6]. We expected Malawian and UK infants to have no or minimal prior mycobacterial exposure and therefore to make similar responses to BCG vaccination. We compared T cell responses induced by BCG vaccination of infants in Malawi and the UK, measuring IFNγ released into supernatants from diluted blood cultures stimulated with M.tb PPD.

Materials and Methods

Recruitment and study design

117 UK infants were recruited at health centers after informed maternal consent. Infants (n=62) who received BCG intradermally (Danish 1331 SSI, 0.05ml) in the first 3-13 weeks of life (median 7 weeks) were recruited in Waltham Forest Primary Care Trust (PCT), and unvaccinated age-matched infants (n=55) from Redbridge PCT. Blood samples were taken from vaccinated infants at three months (12-16 weeks) (n=51) and twelve months (50-56 weeks) (n=38) post-vaccination. Unvaccinated blood samples were taken at age-matched time-points at approximately 6 months of age (n=36) and 15 months of age (n=34).

In Malawi, recruitment took place at Chilumba Rural Hospital, Karonga. After obtaining informed consent and counselling, women were tested for HIV and offered nevirapine if positive. A total of 615 babies were BCG-vaccinated (Danish 1331 SSI, 0.05ml); 590 were bled at 3 months post-BCG and 552 at 12 months post-BCG. Infants were vaccinated between 0 and 198 days of life, 62% (383/615) in the first week of life. A subset of 109 infants vaccinated between 3 and 13 weeks of life were compared with the UK infants, who were vaccinated at the same age. Infants who were born to HIV-positive mothers (7%, 8/109) were excluded.

Sample collection took place between March 2003 and November 2005. Approval for the study was given by the Redbridge and Waltham Forest Health Authority Local Research Ethics Committee, the Ethics Committee of the London School of Hygiene & Tropical Medicine, and by the National Health Sciences Research Committee of Malawi.

Whole Blood Assay and IFNγ ELISA

Whole blood assays and ELISAs for IFNγ were carried out as previously described [5, 7]. Heparinised whole blood was diluted 1 in 10 and cultured on the day of collection with the M.tb PPD (Statens Serum Institut, Copenhagen (SSI), RT49, lot 204) at a concentration of 5μg/ml. PHA-P (Difco Laboratories/Becton Dickinson, Oxford, UK, concentration 5μg/ml) was used as a positive control and medium alone as the negative control. Cultures were incubated at 37°C with 5% CO_2; supernatants were harvested on day 6 and stored at −70°C until assayed for IFNγ in single 100μl samples by quantitative ELISA (detection limit 31pg/ml).

Infants were grouped by season of their birth. In the UK, seasons were autumn (September-November), winter (December-February), spring (March-May), summer (June-August). In Malawi, the seasons were warm/rainy (January-May), cool/dry (June-September), and hot/dry (October-December).

Quality Control

We found no technical factor to explain the differences between the UK and Malawian results (see supplementary material).

Statistical analysis

Data were double entered and verified; and analyzed using STATA 9. Negative control (RPMI) values were subtracted from all IFNγ ELISA results. A positive IFNγ response was defined as >62pg/ml, twice the limit of detection of the assay [8]. The proportion of infants who made an IFNγ response was compared across groups using Chi-square tests. Non-parametric Mann Whitney tests were used to compare IFNγ responses amongst responders.

Results

IFNγ responses to M.tb PPD

In the UK, all vaccinated infants made an IFNγ response to M.tb PPD three months post-vaccination, and 95% twelve months post-vaccination (51/51 and 36/38 respectively). Unvaccinated controls made no IFNγ response to M.tb PPD at either time-point (0/36, 0/34), ($P<0.0001$ comparing vaccinated and unvaccinated infants at either time-point). In Malawi, only 53% of vaccinated infants responded at three months and 48% at twelve months (41/78, 37/77 respectively). The median response in vaccinated infant responders were 1779pg/ml (UK) and 289pg/ml (Malawi) ($P<0.0001$) at three months with a decline in the median response by twelve months to 926pg/ml and 204pg/ml (Fig. 1). In Malawi, 28% of infants did not respond at either time point, 26% responded at only three months, 20% responded at only twelve months, and 26% responded at both time-points. IFNγ responses to M.tb PPD in the Malawian infants who were vaccinated within the first week of life were similar: 64% (135/210) and 43% (114/267) responded at three and twelve months respectively.

IFNγ responses to positive and negative controls

Positive responses to un-stimulated control cultures were rare (<1% UK and <8% Malawi). Positive IFNγ responses to the mitogen PHA were seen in 88% and 74% of vaccinated UK infants three and twelve months post-BCG. In unvaccinated controls, responses were seen in 75% of three month samples, but only 27% of twelve month samples. In Malawi, positive IFNγ responses to PHA were seen in 51% and 45% of vaccinated infants, three and twelve months post-BCG vaccination. There was an association between the Malawian IFNγ responders to M.tb PPD and to PHA at three months post-BCG, with 23/38 (62%) of PHA non-responders also being PPD non-responders, and 14/40 (38%) of PHA responders not responding to PPD ($P=0.024$). This trend was also observed at twelve months post-BCG ($P=0.055$). When restricted to PHA responders, the proportion of M.tb PPD responders in Malawi was much lower (63% and 57% responded at three and twelve months post-BCG) than in the UK (100% and 95% responded at three and twelve months post-BCG) ($P<0.0001$).

Skin test responses and scar sizes

Mantoux skin tests were performed in Malawian infants 3 months post-BCG vaccination. Forty nine percent (37/76) of infants made no skin test response (0mm), while 51% percent made a skin test response. Of these infants, 26% (20/76) made a response between 1 and 9mm and 25% (19/76) made a response above 10mm. There is a strong association between skin test response and IFNγ measured in supernatants stimulated with M.tb PPD ($P<0.0001$). The median BCG scar size 3 months post-BCG vaccination in the UK (5mm) was larger than in Malawi (3mm) ($P<0.0001$), with 100% of UK infants forming a scar while only 80% of Malawian infants made a scar.

Seasonal variation in responses

In Malawi, infants who were born in the hot/dry season were more likely to make an IFNγ response (>62pg/ml) to M.tb PPD three months post-BCG vaccination (10/14, 71%) than infants born in the cool/ dry season (12/22, 55%), who were more likely to make a response than infants born in the warm/rainy season (19/42, 45%) (P=0.230) (Fig. 2a).

There were more high responders (>500pg/ml) in infants born in the hot and dry season (P=0.002) (Fig. 2a). The median IFNγ response to M.tb PPD among responders also varied by season (206pg/ml in the warm/rainy season, 240pg/ml in the cool/dry season, and 1729pg/ml in the hot/dry season) (Fig. 2b).

As the numbers of Malawian infants in this restricted data set were small, the full dataset (615 infants) was also analysed. Three months post-vaccination, the odds of making a positive IFNγ response were higher in the hot/dry, and cool/dry seasons than in the warm/ rainy season (Odds ratio= 5.1, P<0.001 and Odds ratio=1.8, P=0.04, respectively). Twelve months post-vaccination, the trend was in the same direction, although weaker (Odds ratio=2.1, p=0.005 and Odds ratio=1.3, p=0.28 respectively). The trend was similar when using a high (>500pg/ml) cut off for an IFNγ responder, and was seen in each of the two years of the study (data not shown). IFNγ responses to M.tb PPD by season of BCG vaccination instead of birth gave similar results.

In the UK, median responses were higher in infants who were born in the spring (2078pg/ml) and summer (1922pg/ml) than those born in the autumn (1342pg/ml) and winter (1196pg/ml) (Fig 2a, b) (P=0.04).

Birthweights

Only 7% of Malawian infants were considered low birthweight, and birthweight did not confound the association between birth season and IFNγ response. In Malawi, there was a weak association between birthweight and IFNγ response to M.tb PPD at 3 months (P=0.116), but not at 12 months (P=0.6).

Discussion

In this study, infants responded differently to BCG in the UK and Malawi. While all vaccinated infants in the UK produced a strong IFNγ response three months post-BCG to M.tb PPD, only 53% of infants in Malawi were IFNγ responders, and responses were of a lower magnitude than UK infants. The proportion of IFNγ responders to M.tb PPD observed three months post-BCG was maintained to twelve months in both the UK and Malawi, while the median response decreased in the UK but not Malawi. There was greater variability in response in Malawian infants, with 46% responding at only one time-point. In Malawi, only 47% of infants made a skin test response of >5mm three months post-BCG, although this is higher than was seen two months post-BCG in Guinea Bissau (39% above 1mm) [9]. Scars were present at 3 months post-BCG in only 80% of Malawian infants, and were smaller than the median size of scar in the UK. Unvaccinated controls in the UK did not produce any detectable IFNγ response to M.tb PPD at either time-point.

Differences observed in the responses to BCG vaccination in UK and Malawian adolescents have been attributed to the high proportion of Malawian individuals already exposed and sensitised to a variety of environmental mycobacterial antigens prior to vaccination [6]. Masking or blocking of the BCG response through exposure to environmental mycobacteria remains a plausible explanation, although we were unable to demonstrate a relationship between prior IFNγ responses to specific environmental mycobacteria antigens, and BCG-attributable changes in IFNγ to M.tb PPD in UK or Malawian adolescents [10]. The

differences observed in the UK and Malawian infants are unlikely to be due to prior environmental exposure, as Malawian infants vaccinated in the first week of life had low IFNγ responses to M.tb PPD.

There has been no formal evaluation of neonatal BCG protection against childhood forms of tuberculosis in Malawi. Given the large population differences observed between the immune responses to BCG vaccination in the UK and Malawian infants it is possible that BCG vaccination does not offer equal protection to infants in different countries. Studies of neonatal BCG in some other African countries, such as South Africa and The Gambia, have shown strong IFNγ responses induced in similar assays. In The Gambia, IFNγ responses to M.tb PPD measured in PBMC cultures were similar to adult responses [11]. In South Africa, IFNγ was measured by flow cytometry following stimulation of whole blood with live BCG, ten weeks post-vaccination [12]. However a randomised trial in Guinea Bissau, examining the effect of Vitamin A supplementation on immune response to BCG using a similar whole blood assay to ours found comparably low median IFNγ responses (199pg/ml to M.tb PPD), similar to the Malawian responses in our study [9].

IFNγ responses to PHA were lower than expected at three and twelve months post-BCG in UK and Malawian infants. In the UK, whereas 100% of vaccinated infants made a response to M.tb PPD three months post-BCG, only 88% of these infants made a response to PHA. In Malawi, responses to PHA were low, with only 51% of the infants responding three months post-BCG. In future studies Staphylococcal enterotoxin B (SEB) [12] may be a better positive control to use in infants than 5ug/ml PHA.

IFNγ responses to M.tb PPD three months post-BCG varied with the season of birth of the infant. In Malawi, the proportion of IFNγ responders was higher in infants born during the hot/dry season, and the median response amongst responders was similar to the median response in the UK. In the UK, a difference in IFNγ response to M.tb PPD by season of birth was also observed, with greater responses in the summer. Seasonal variation in responses to rabies and typhoid vaccinations have been reported in the Gambia and Pakistan [13], while a recent study of BCG-vaccinated infants in The Gambia showed higher proportions of CD4 T cells expressing CD154 at 12 months of age in infants born in the wet compared to the dry season [14]. Our observed seasonal variation in IFNγ responses to BCG vaccination suggest that environmental factors play a role in vaccine induced immunity. These could include seasonal differences in the burdens of infections, such as malaria or respiratory infections in the wet season in Malawi, and common respiratory infections in the winter in the UK. The varying responses in Malawi could also be due to nutritional factors which vary by season depending on availability of food.

Other factors which may also be influencing the different responses seen in Malawian and UK infants include differences in the maturation of the immune system, T cell clonal responses[15], burdens of maternal infectious diseases or schedules of other vaccinations. This is the first study which shows that population differences in responses to BCG vaccination occur in infants as well as adults. This finding has important implications for the use of BCG and the development and testing of future TB vaccines designed to boost immune responses induced by BCG. It may also have implications for immune responses to other infant vaccinations, and warrants further study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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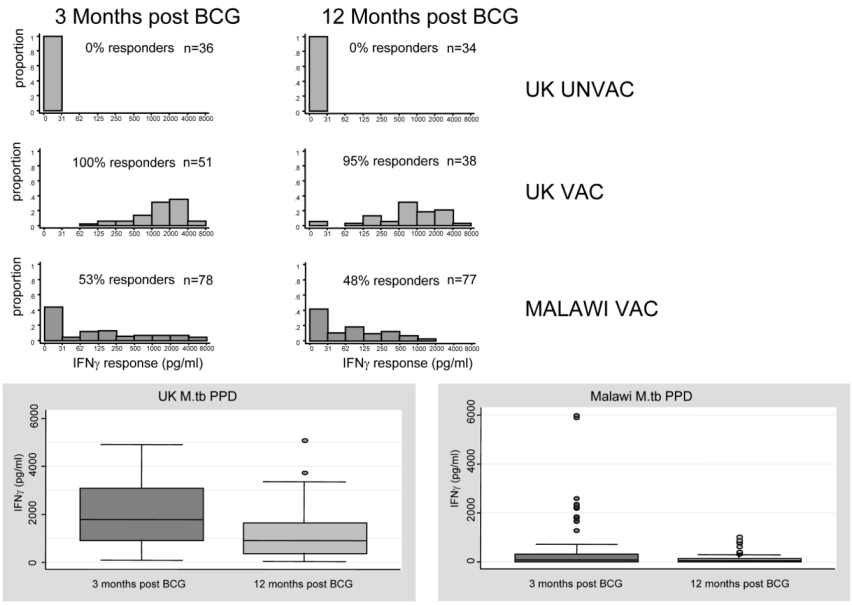

Figure 1. IFNγ responses to M.tb PPD

a) Histograms showing IFNγ responses to M.tb PPD in unvaccinated UK infants, vaccinated UK infants, and vaccinated Malawian infants three and twelve months post BCG vaccination and age-matched for unvaccinated controls. **b)** Box and whisker plots showing IFNγ responses to M.tb PPD in vaccinated UK and Malawian infants three and twelve months post BCG vaccination.

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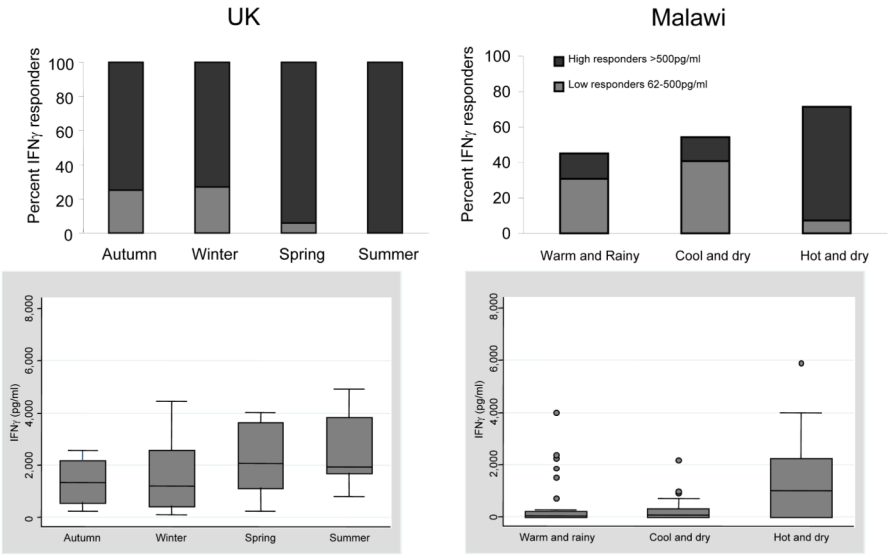

Figure 2. IFNγ responses to M.tb PPD three months post BCG vaccination by season of birth

a) Bar charts showing percentage of low responders (62-500pg/ml) and high responders (>500pg/ml) by season in the UK and Malawi. In the UK, responses are shown for Autumn (Sept, Oct, Nov), Winter (Dec, Jan, Feb), Spring (Mar, Apr, May), Summer Jun, Jul, Aug). In Malawi, seasons are warm and rainy (Jan, Feb, Mar, Apr, May), cool and dry (Jun, Jul, Aug, Sep), hot and dry (Oct, Nov, Dec).

b) Box and whisker plots showing IFNγ responses to M.tb PPD in Malawi and the UK by season. (UK n=51, Malawi n=78).