The immunological effects of oral polio vaccine provided with BCG vaccine at birth: A randomised trial

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A B S T R A C T

Background: Vaccines may have non-specific effects. An observational study from Guinea-Bissau suggested that oral polio vaccine at birth (OPV0) provided with Bacillus Calmette–Guérin (BCG) vaccine was associated with down-regulation of the immune response to BCG vaccine 6 weeks later. Based on the previous finding, we wanted to test our a priori hypothesis that OPV would dampen the immune response to BCG, and secondarily to test immune responses to other antigens.

Methods: The study was conducted at the Bandim Health Project in Guinea-Bissau in 2009–2010. Infants were randomised to OPV0 + BCG versus BCG alone at birth, and subsequently randomised to have a blood sample taken at 2, 4 or 6 weeks post-randomisation. Excreted levels of cytokines (IL-2, IL-5, IL-10, TNF-α and IFN-γ) and IL-5, IL-10, IL-12, and IFN-γ interferonγ secreted into whole blood in vitro stimulations with a panel of recall vaccine antigens (BCG, PPD, OPV0, mitogen (PHA) or innate agonists (LPS, Pam3cys, PolyI:C). Additionally, we measured the local reaction to BCG, white blood cell distribution, C-reactive protein (CRP) and retinol-binding protein (RBP). Cytokine production was analysed as the prevalence ratios of responders above the median.

Results: Blood samples from 430 infants (209 OPV0 + BCG; 221 BCG alone) were analysed. There were no strong differences in effects 2, 4 and 6 weeks post-randomisation and subsequent analyses were performed on the pooled data. As hypothesised, receiving OPV0 + BCG versus BCG alone was associated with significantly lower prevalence of IFN-γ responses to PPD (prevalence ratio (PR): 0.84 (0.72–0.98)) and reduced IL-5 to PPD (PR: 0.78 (0.64–0.96)). No effects were observed for CPR, RBP, white blood cell distribution, or BCG scar prevalence.

Conclusion: The results corroborate that OPV attenuates the immune response to co-administered BCG at birth.

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1. Introduction

According to current vaccination policy, infants in high-risk countries should receive oral polio vaccine at birth (OPV0) followed by three doses in infancy [1]. The first dose at birth is usually given together with Bacillus Calmette-Guérin vaccine (BCG) against tuberculosis (TB). Recently, OPV was temporarily missing in Guinea-Bissau. In this "natural experiment", not receiving OPV0 was associated with increased infant male survival but a weak tendency for increased mortality among females, indicating that OPV0 may have a sex-differential effect on infant mortality.
The BCG given at birth is known to induce a potent pro-inflammatory Th1-polarising IFN-γ response to purified protein derivative from Mycobacterium tuberculosis (PPD) [3]. However, in the “natural experiment” receiving OPV0 with BCG at birth was associated with significantly lower IFN-γ in response to PPD at 6 weeks of age, and a moderately lower likelihood of developing a BCG scar, suggesting that OPV0 may dampen the response to BCG [4]. It could be speculated that part of the lower BCG vaccine efficacy in low-income countries [5] might be due to simultaneous OPV0.

To further investigate the heterologous effects of OPV, we carried out a large randomised-controlled trial (RCT) testing the effects of providing OPV with BCG at birth on overall survival (Lund, submitted). The present sub-study aimed at investigating the immunological effects of OPV together with BCG at birth on the developing immune response at 2, 4 and 6 weeks of age, including innate and non-polio specific adaptive responses, non-specific inflammation markers and immune cell distribution. Our a priori hypothesis was that OPV would dampen the IFN-γ response to PPD.

2. Methods and materials

2.1. Setting

The present immunological study was carried out within a larger RCT investigating the effects of providing OPV0 with BCG at birth on infant survival. The trial was conducted from July 2008 to October 2011 at the Bandim Health Project (BHP), a health and demographic surveillance system site covering six suburban districts of Bissau, the capital of Guinea-Bissau, West Africa.

2.2. Enrolment into the main trial

The trial has been described elsewhere (Lund, submitted; clinicaltrials.gov: NCT00710983). In brief, newborns with no overt illness or malformations, weighing ≥2.5 kg at enrolment and living in the BHP study area were eligible for recruitment. Mothers received oral and written information. Provided consent, the mother drew a randomisation number allocating her infant to receive OPV0 together with the BCG (OPV0+BCG) or BCG alone (BCG). The BCG (Danish strain 1331, Statens Serum Institut, Copenhagen, Denmark) was given intra-dermally in the upper left deltoid region while the trivalent OPV was administered as two drops orally.

2.3. Enrolment into the present sub-study

From 27 May 2009 to 7 April 2010, infants delivered on weekdays at the maternity ward at the Simão Mendes National Hospital and randomised within the first 7 days of life were invited to participate in the present immunological sub-study, excluding infants delivered by caesarean section or twins. During the synchronised West African Polio Immunisation Campaigns in March and April 2010 some infants were not included (n = 32) (Fig. 1). Informed consent was obtained according to the same procedure as the main trial.

Measurements of weight, length, circumferences of abdomen, head and mid-upper-arm and axillary temperature of the infant, and axillary temperature of the mother were obtained at enrolment. Subsequently, the infants were randomised to a follow-up visit at home at 2, 4 or 6 weeks after enrolment. Infants who received other vaccines before blood sampling were excluded from the study (Fig. 1).

2.4. Follow-up

At the follow-up visit at 2, 4 or 6 weeks a blood sample was collected, the mother was interviewed about the health of her infant; the mid-upper-arm circumference and axillary temperature of the infant were measured; formation of scar or local reaction at the site of BCG vaccination was recorded (yes or no).

Additionally, the main trial also recorded the presence and size of BCG scar at 2, 6 and 12 months after enrolment on the same infants. Size of the scar was measured as the average of length and width measured to nearest millimetre with a transparent ruler.

2.5. Blood sampling and whole blood stimulations

At enrolment, a pre-vaccination baseline dried blood spot (DBS) on filter paper was collected by heel prick puncture for measurement of retinol-binding protein (RBP) and C-reactive protein (CRP). The filter paper was dried in up-right position overnight and stored with silica desiccant at −20°C until analysis. At the follow-up visits, capillary blood was collected by heel puncture into a heparinised tube for whole-blood stimulation and in an EDTA-coated tube for differential counts, respectively. A DBS for RBP and CRP measurements was collected similarly to the baseline. A blood smear was microscopically inspected for malaria parasites. From collection to processing, the heparinised blood was kept at ambient temperature; the EDTA-treated blood was kept cold. All blood samples were collected by the same trained nurse and transported to the National Laboratory within 4 h. The whole blood stimulation assay was performed as previously described [6,7]. Briefly, the heparinised blood was diluted 1:10 with RPMI-1640 medium (Invitrogen, Breda, Netherlands) supplemented with 2 mM glutamate, 1 mM pyruvate, 100 IU penicillin and 100 μg/ml streptomycin, and cultured at 37°C with 5% CO2, supplemented with lipopolysaccharide (LPS) (1 ng/ml, Sigma-Aldrich, Zwijndrecht, Netherlands) [a Toll-like receptor (TLR) agonist], (S)-(2,3-bis(palmitoyloxy)-(2-RS)-propyl)-(2-RS)-propyl-N-palmitoyl-(R)-Cys-(S)-Ser-(S)-Lys-4-OH, trihydrochloride (Pam3cys) (100 ng/ml, Cayla-InviGo/EN Europe, Toulouse, France) [a TLR2 agonist], antigen purified protein derivative (PPD) of Mycobacterium tuberculosis (10 μg/ml, Statens Serum Institut, Copenhagen, Denmark), BCG (Statens Serum Institut, final concentration 1:100), trivalent OPV (final concentration 1:100) or phytohaemagglutinin (PHA) (2 μg/ml, Welcome Diagnostics, Dartford, UK) [a T cell mitogen]. Controls were medium alone cultures (referred as medium). Supernatants were collected after one day (for LPS, Pam3cys and medium1) or three days of incubation (for PPD, BCG, OPV, PHA, poly I:C and medium3) and stored below minus 40°C until cytokine measurements.

2.6. Measurement of cytokine concentrations

Cytokine concentrations in supernatants were analysed at Statens Serum Institut, Copenhagen, Denmark. IL-10 and TNF-α from day 1 supernatants stimulated with LPS and Pam3cys, and IL-2, IL-5, IL-10, TNF-α and IFN-γ from day 3 supernatants stimulated with PPD, BCG, OPV, PHA and poly I:C were analysed using Luminex cytokine kit and buffer reagent kit (BioSource, Camarillo, CA, USA) on a Luminex-200 cytometer (Luminex Corporation, Austin, TX, USA) equipped with Bio-Plex Manager version 5.0 (Bio-Rad, Hercules, CA, USA). The assay was performed according to the manufacturer’s instructions with slight modifications. Briefly, assays were performed in a 96-well U plate (NUNC, Roskilde, Denmark) at room temperature. Mixes of beads were incubated for 2 h with the test samples, the standard dilutions
series, inter-assay controls and a blank in a final volume of 50 μl for 2 h under continuous shaking. Beads were washed twice and incubated with biotinylated antibodies (25 μl/well) for 1 h. After removal of excess antibodies, streptavidin-PE was added for 30 min. The plate was then washed and analysed. The lower detection limits of the assay defined by the manufacturer were 6, 3, 5, 5 and 10 pg/ml for IL-2, IL-5, IL-10, IFN-γ and TNF-α, respectively.

2.7. Differential counts

Differential counts were performed on EDTA-treated blood by using ABX Pentra 60 Hematology Analyzer (Horiba Diagnostic Group, France). Due to logistic challenges in the laboratory, haematological analyses were only conducted on blood samples collected after 24 October 2009. Samples with an improper separation and gating of the detected cell subsets as assessed by visual inspection...
of the scatter plot produced by the ABX Pentra60 were repeated if sufficient amount of blood was available; poor quality analyses were excluded.

2.8. Measurements of RBP and CRP

From the DBSs, RBP and CRP were measured concurrently by a combined simple sandwich ELISA method [8,9]. The samples were tested in duplicates with the paired baseline and follow-up samples in the same assay. Samples with a coefficient of variance >20% were repeated in duplicates.

2.9. Statistical analysis

Data was analysed using STATA 12 (StataCorp LP, College Station, TX, USA).

As in our previous study [4], cytokine outcomes were categorised as below versus above the median, and analysed by Poisson regression with robust estimate variance providing prevalence ratios (PR) of being above the median in OPV0 + BCG versus BCG alone recipients. The prevalence of BCG scars or local reactions was analysed by Poisson regression with robust estimate variance. BCG scar size was analysed by linear regression.

For every plate analysed on the Luminex instrument, the range of the cytokine analysis assay was defined by the lower and upper range of the standard series after censoring for standard concentrations outside a recovery limit of 80–120% (observed concentration versus expected concentration). If the lower detection limit as defined by the manufacturer was higher than the lower limit inferred from the standard series, the former was applied. Observations outside this range were considered as non-detectable.

Cytokine outcomes with >50% detectable measurements were log-transformed and analysed with Tobit regression to account for observations below or above the detection range of the Luminex assay [10]. The estimates were back-transformed to give the geometric mean ratios (GMR) comparing OPV0 + BCG with BCG alone. Hence, a GMR or a PR > 1 may be interpreted as OPV increasing

Table 1
Background characteristics of study population.

<table>
<thead>
<tr>
<th>Part A. At randomisation</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>209</td>
<td>102</td>
<td>107</td>
</tr>
<tr>
<td>Males</td>
<td>102 (49%)</td>
<td>53 (54%)</td>
<td>50 (53%)</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>206 (99%)</td>
<td>103 (99%)</td>
<td>103 (99%)</td>
</tr>
<tr>
<td>Age &gt;1 day</td>
<td>15 (7%)</td>
<td>7 (7%)</td>
<td>8 (7%)</td>
</tr>
<tr>
<td>Mother has been to school</td>
<td>162 (82%)</td>
<td>81 (81%)</td>
<td>81 (81%)</td>
</tr>
<tr>
<td>Electricity in house</td>
<td>95 (46%)</td>
<td>47 (47%)</td>
<td>48 (48%)</td>
</tr>
<tr>
<td>Toilet is outdoor</td>
<td>166 (81%)</td>
<td>83 (83%)</td>
<td>83 (83%)</td>
</tr>
<tr>
<td>Rainy season</td>
<td>86 (41%)</td>
<td>43 (43%)</td>
<td>43 (43%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Part B. At follow-up</th>
<th>All</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>209</td>
<td>102</td>
<td>107</td>
</tr>
<tr>
<td>Follow-up at 2 weeks</td>
<td>75 (36%)</td>
<td>38 (38%)</td>
<td>37 (37%)</td>
</tr>
<tr>
<td>Follow-up at 4 weeks</td>
<td>75 (36%)</td>
<td>38 (38%)</td>
<td>37 (38%)</td>
</tr>
<tr>
<td>Follow-up at 6 weeks</td>
<td>59 (28%)</td>
<td>29 (29%)</td>
<td>30 (29%)</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>209 (100%)</td>
<td>104 (100%)</td>
<td>105 (100%)</td>
</tr>
<tr>
<td>Symptoms</td>
<td>60 (29%)</td>
<td>30 (30%)</td>
<td>30 (30%)</td>
</tr>
<tr>
<td>Fever</td>
<td>29 (14%)</td>
<td>15 (15%)</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>Cough</td>
<td>43 (21%)</td>
<td>22 (22%)</td>
<td>21 (21%)</td>
</tr>
<tr>
<td>Is doing well</td>
<td>190 (91%)</td>
<td>95 (95%)</td>
<td>95 (95%)</td>
</tr>
<tr>
<td>In vitro cytokines</td>
<td>181 (87%)</td>
<td>90 (90%)</td>
<td>91 (91%)</td>
</tr>
<tr>
<td>Differential counts</td>
<td>103 (49%)</td>
<td>52 (52%)</td>
<td>51 (51%)</td>
</tr>
<tr>
<td>CRP/RBP measurements</td>
<td>195 (93%)</td>
<td>97 (97%)</td>
<td>96 (96%)</td>
</tr>
</tbody>
</table>

| MUAC (mm)             | 25 (18–34) | 24 (18–33) | 25 (18–34) |
| Temperature (°C)      | 36.7 (0.3) | 36.7 (0.3) | 36.7 (0.3) |

Characteristics of the study population at baseline (A) and follow-up (B). Symptoms: fever or cough as reported by the mother or an axillary temperature >37.5 °C. MUAC: mid-upper-arm circumference, used as a marker of nutritional status.

For categorical values, statistical test for difference between randomisation groups by χ² test, alternatively Fisher’s exact test for outcome with levels of ≤5 observations. For continuous values, test by student’s t-test for normally distributed values, or Kruskal–Wallis for non-normal values. Median is presented with 10 and 90 percentiles. Mean values is presented with standard deviation (sd).

In vitro stimulations were only performed after the 18 August 2009. EDTA-treated blood was only collected after the 7 October 2009. Differential counts were commenced only from 24 October 2009.
the given outcome. Log-transformed haematological data was analysed with linear regression using bootstrap to obtain confidence intervals (CI). CRP and RBP were analysed by Poisson regression as the risk of having a CRP measurement >5 μg/ml or a RBP level <0.83 μmol/l (vitamin A-deficient [11]). RBP was log-normally distributed and analysed by linear regression. Analyses of follow-up CRP and RBP were adjusted for the respective baseline values.

As the previous observational study had suggested sex-differential effects of OPV on mortality [2], all analyses were stratified by sex and follow-up at 2, 4 or 6 weeks, including a test of effect modification on the OPV effect of both sex and follow-up time. When analysing all follow-up groups combined, follow-up time was adjusted for.

We aimed at enrolling 400 infants (200 OPV+BCG; 200 BCG) in the immunological study based on preliminary data from the “natural experiment” [4] indicating a significant reduction in the IFN-γ responses to PPD in children receiving OPV0 (n = 250) versus no OPV0 (n = 150).

3. Results

In total, 611 newborns enrolled in the main trial were eligible for inclusion in the immunological sub-study. Of these, 461 infants had a follow-up blood sample taken; valid in vitro cytokine analyses were performed on 378 infants, valid differential counts were available for 212 infants, and paired baseline and follow-up measurements of RBP and CRP were obtained from 404 infants (Fig. 1). The two randomisation groups (OPVO+BCG versus BCG) did not differ at baseline, except for a slightly, but significantly higher mean temperature in the OPV0+BCG group (Table 1). At follow-up, the two randomisation groups were similar in respect to disease symptoms and nutritional status (Table 1). No parasitaemia was found. Overall, the participants included in the immunological analyses were similar to the study population enrolled in the main RCT (data not shown).

Blood samples were collected at 2, 4 or 6 weeks after randomisation. For most of the cytokine outcomes, there was a significant effect of follow-up time, in most cases there were increased cytokine responses with increasing time since vaccination (data not shown). However, the effect of OPV0 was not significantly different at the three follow-up time points (Supplementary Table 1). For all responses to PPD and BCG except IL-10, the difference between infants vaccinated with OPV0 + BCG versus BCG alone was most pronounced at 4 weeks after randomisation, although the difference was small in absolute terms (Fig. 2 and Supplementary Table 1). Hence, we merged the data, and subsequently analysed the effect of OPV0 + BCG versus BCG alone adjusting for follow-up time.

Fewer children who received OPV0 + BCG versus BCG alone had a high IFN-γ and IL-5 response to PPD (prevalence ratio (PR): 0.84 (95% CI: 0.72–0.98) and 0.78 (0.64–0.96), respectively) (Table 2). Analysed as continuous data, the response IL-5 to PPD was significantly lower (geometric mean ratio (GMR) of 0.70 (0.51–0.97) (Supplementary Table 2).

For non-specific cytokine responses, there was no difference between infants vaccinated with OPV0 + BCG versus BCG alone (Table 2 and Supplementary Table 2). Overall, the effect of OPV0 + BCG was not modified by sex of the infant apart from the IL-2 responses to PHA, where OPV0 + BCG was associated with a reduced prevalence of high responses in males only (Table 2).

We also analysed the effect of OPV0 + BCG on ratios of IFN-γ to IL-5 (Th1 versus Th2) and TNF-α to IL-10 (pro- versus anti-inflammatory) for outcomes with >50% detectable measurements. OPV0 + BCG did not affect these ratios (data not shown).

OPV0 + BCG were not associated with the prevalence of having a BCG scar or local reaction at follow-up, or at 2, 6 and 12 months of age. There was no difference in the size of scars. At 12 months, all infants had developed a BCG scar (Table 3).

OPV0 + BCG was associated with a trend for higher neutrophil counts (GMR: 1.15 (1.01–1.31)). Other haematological values were not affected (Supplementary Table 3).
4. Discussion

As hypothesised, co-delivery of OPV with BCG at birth reduced the IFN-γ response to BCG vaccination. Also IL-5 responses to PPD were reduced by OPV. We found no effect on BCG scarring; at 12 months, all infants had developed a scar. OPV was associated with higher neutrophil counts, but no effects on CRP or RBP levels were observed. The study is the first RCT demonstrating a heterologous immunological effect of OPV0.

5. Strengths and limitations

The trial design allowed us to investigate the effect of OPV + BCG versus BCG alone in an unbiased manner. The participants in the present immunological investigation were a representative sub-group of the overall study population.

Whereas the previous observational immunological study of OPV0 was constrained by comparing OPV0 + BCG to BCG in the rainy season only [4], the present investigation enrolled infants over almost a year covering both the rainy (June to November) and the dry (December to May) season.

The hypothesis in relation to the immune response to BCG was pre-specified and it should not be necessary to adjust for multiple testing. However, the other analyses were exploratory and should therefore be interpreted with appropriate caution. No placebo was used in the study. However, the technicians processing the samples were blinded to the randomisation.

6. Previous studies

Preliminary results from the main trial show that receiving OPV0 was not associated with increased infant mortality, and there was no significant difference in males versus females. Intriguingly, the effect depended on the age at enrolment; for children enrolled within the first 2 days of life, the hazard ratio for BCG alone versus OPV0 + BCG was 1.71 (1.11–2.64), while it was 0.82 (0.52–1.30) for children enrolled at ≥3 days (p for interaction = 0.02) (Lund, submitted). This stratification could not be performed in the immunological study, however, as too few infants were enrolled beyond 2 days. Whether the protective effect of early delivery
Table 3

<table>
<thead>
<tr>
<th>Sex</th>
<th>OPV + BCG vs BCG</th>
<th>OPV vs BCG</th>
<th>BCG alone</th>
<th>OPV + BCG vs BCG</th>
<th>OPV vs BCG</th>
<th>BCG alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scar freq</td>
<td>Male</td>
<td>PR for no scar</td>
<td>n (%)</td>
<td>PR for no scar</td>
<td>n (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-6 Weeks</td>
<td>174 (89%)</td>
<td>180 (90%)</td>
<td>0.81</td>
<td>1.00</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>6 Months</td>
<td>173 (100%)</td>
<td>180 (100%)</td>
<td>0.52</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>12 Months</td>
<td>164 (100%)</td>
<td>180 (100%)</td>
<td>0.52</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Scar size</td>
<td>Mean (sd)</td>
<td></td>
<td>Mean (sd)</td>
<td></td>
<td>Mean (sd)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4 (1.1)</td>
<td></td>
<td>4.5 (1.1)</td>
<td></td>
<td>4.5 (1.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1 (0.7)</td>
<td></td>
<td>4.2 (0.7)</td>
<td></td>
<td>4.2 (0.7)</td>
<td></td>
</tr>
</tbody>
</table>

Presence of scar included children who received OPV + BCG at birth and BCG alone at 2–6 weeks. The PR is adjusted for follow-up time.

of OPV0 may be mediated via a beneficial immune-programming could not be corroborated with the immunological markers we here investigated.

OPV may reduce, albeit non-significantly, rota virus titres and sero-conversion rate when co-administered with live rota virus vaccine [12]. A transient suppressive effect of OPV (Sabin type 1) on tuberculin reactivity was observed decades ago in TB-infected children receiving chemotherapy. However, OPV did not impair the clinical remission of the TB infection [13]. Recently, a “natural experiment” from Bissau found that OPV0 was associated with reduced in vitro IFN-γ responses to PPD 6 weeks after co-administration with BCG and lower likelihood of developing a BCG scar at 2 months [4]. A later similar observational study found that OPV0 was associated with fewer BCG scars for males but not for females at 2 months of age [14].

In the present study, virtually all infants have developed a scar after 6 months, and the size of the local reaction did not differ between the randomisation groups. The very high scar rate was higher than the rates reported previously for both BCG + OPV and BCG alone [4] and may reflect that all infants were BCG vaccinated by trained nurses at the national hospital with long experience [15].

The results of the present study confirm the previous observation that OPV attenuates the in vitro responses to PPD, as the frequency of high IFN-γ responders and the production of IL-5 to PPD were reduced in infants receiving OPV + BCG. Hence, OPV was associated with a non-biased attenuation of both Th1 and Th2 skewing cytokine responses.

Of note, OPV was not found to induce leukopenia or lymphocytopenia. The observed association of OPV with neutrophil counts has not been described previously and should be tested in another study.

The in vitro cytokine responses to OPV stimulation were at similar or lower levels than the control samples, which is in line with our previous experiences with the assay (unpublished data). Relatively low infant cellular responses to polio-antigen have been reported previously [16]. OPV stimulation may have had an inhibitory effect during the incubation. OPV-infected dendritic cells (DC) are impaired in receptor-mediated endocytosis [17], and it has been suggested that DC infected with polio are impaired in the MHC class I expression [18], although this has been contradicted in a later study [17]. The putatively inhibitory effect of OPV in culture may parallel the observed attenuation of BCG responses. Notably, the immunological interaction is systemic as OPV and BCG are administered via different routes (oral versus intra-dermal).

The protective effects of BCG against TB is generally lower in low-income countries [5], and geographical differences in the immunological effects of BCG has been observed [19]. It could be speculated that OPV may contribute to this attenuation of the BCG effects. Although disputed [20], in vitro IFN-γ responses to PPD is a widely used marker of TB immunity [21]. It is not known, however, how the magnitude of the response early in life is associated with increased susceptibility later in life [22]. In conclusion, the present study corroborates that OPV may have non-specific effects, as OPV was associated with a reduced immune response to BCG.

Conflicts of interest statement

None.

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Research to CSB. PA holds a research professorship grant from the Novo Nordisk Foundation. CSB was funded by an ERC Starting Grant (ERC-2009-StG-243149). CVIVA is funded by the Danish National Research Foundation (DNRF108). The Bandim Health Project received support from DANIDA. The funding agencies had no role in the study design, data collection, data analysis, data interpretation, or the writing of the manuscript.

Author contribution
CSB, PA, NL conceived and designed the study. HSK, NL, AGB, HBE supervised the field work; HSK performed the laboratory analyses; BK supervised cytokine measurements; KJJ analysed the data; AA supervised the data analyses; HSK and KJJ wrote the first draft; all authors contributed to the final version of the paper.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.08.062.

References