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Short Communication

# Impact of Bacille Calmette-Guérin revaccination on serum IgE levels in a randomized controlled trial

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## Abstract

**Introduction:** Bacille Calmette-Guérin (BCG) downmodulates allergen-specific IgE levels and prevents other atopic responses in experimental models but fails to protect against respiratory allergies. Human responsiveness to BCG is variable and may interfere with protection. **Methods:** Multivariate models were evaluated to test the possible effect of responsiveness (assessed by IFN-γ production) to BCG revaccination on the modulation of total and allergen-specific serum IgE levels in healthy volunteers participating in a randomized controlled trial. **Results:** Serum total or Derp-specific IgE levels did not change regardless of the increase in IFN-γ levels. **Conclusions:** BCG responsiveness does not affect protection against atopy.

**Keywords:** Allergy. Atopy. Tuberculosis vaccine.

Studies in humans show no evidence that immunization with *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) protects against allergic diseases[1-3]. Nevertheless, there is increasing evidence that BCG vaccination modulates several aspects of atopy in experimental models of asthma and humans[4]. Mice immunized with BCG and sensitized intranasally with ovalbumin (OVA) had a lower percentage of eosinophils in the bronchoalveolar lavage, reduced levels of total and allergen-specific serum immunoglobulin E (IgE), decreased levels of T-helper 2 (Th2) cytokine, decreased lung inflammation, as well as increased levels of interferon gamma (IFN-γ), tumor necrosis factor beta (TGF-β), and interleukin-10 (IL-10)[5]. Increased levels of allergen-specific serum IgE and eosinophilia are hallmarks of atopic asthma, and the former is correlated with the severity of clinical symptoms[6]. A limited modulation of the immune response by the BCG vaccine in humans may partially explain the discrepancies in the findings of studies using experimental models and humans[7].

In Salvador, Bahia, Brazil, neonatal BCG has been shown to protect children with respiratory allergies and sneezing against asthma[8]. However, children's responses to BCG revaccination are variable, and the levels of IFN-γ and IL-10 are increased in response to mycobacterial antigens *in vitro* in only a few individuals[9].

Here, we investigate the immune modulation elicited by revaccination with BCG in a previously conducted clinical trial that evaluated the in vitro responses to mycobacterial challenge in a sample of healthy BCG-revaccinated volunteers negative for TB infection[10]. We hypothesized that BCG revaccination differentially modulated serum IgE levels in individuals with the high levels of IFN-γ compared with those in which the effects of the vaccine were modest. This hypothesis was tested as a secondary objective of the clinical trial.

### Ethical considerations

This study was approved by the Institutional Review Board of *Instituto Gonçalo Moniz* (CAAE-0010.0.225.069-09) and was registered in the Brazilian Clinical Trials Registry (primary ID No. RBR-2gv984). We analyzed the serum samples from 75 volunteers who participated in a previous randomized, open, controlled (2-arms) clinical trial, of which 46 subjects received BCG revaccination and 29 received no intervention (**Table 1**)[10]. All subjects were healthy undergraduate students with a negative tuberculin skin test upon repeated administration of protein purified derivative (PPD)[10]. The serum samples of two volunteers (one revaccinated and one control) were not analyzed. The Consolidated Standards of Reporting Trials (CONSORT) 2010 statement guidelines were followed, and the study's

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**TABLE 1:** Sociodemographic and clinical characteristics of the study subjects.

| | Revaccinated (n=46)[a] | | Controls (n=29)[b] | |
|---|---|---|---|---|
| | **atopic (n =29)** | **non-atopic (n =16)** | **(atopic n =17)** | **non-atopic (n =11)** |
| Age in years (95% CI)[c] | 20 (19-21) | 20 (19-21) | 20 (17-23) | 20 (18-22) |
| Gender (%) | | | | |
| male | 9 (31.0) | 2 (12.0) | 5 (29.0) | 4 (36.0) |
| female | 20 (69.0) | 14 (88.0) | 12 (71.0) | 7 (64.0) |
| Family income > 5 MW[d] (%) | 22 (76.0) | 11 (69.0) | 13 (76.0) | 8 (73.0) |
| n/a | 1 (3.0) | 0 (0.0)0 | 1 (6.0) | 0 (0.0) |
| Smoking (%) yes | 2 (7.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| n/a | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Drug use (%) | | | | |
| alcohol[e] | 8 (28.0) | 6 (38.0) | 1 (6.0) | 3 (27.0) |
| other[f] | 1 (3.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| n/a | 3 (10.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Parasitic disease | | | | |
| yes | 3 (10.0) | 1 (6.0) | 2 (12.0) | 0 (0.0) |
| no | 13 (45.0) | 8 (50.0) | 8 (47.0) | 5 (45.0) |
| n/a | 13 (45.0) | 7 (44.0) | 7 (41.0) | 6 (55.0) |
| BMI[g] (95% CI)[c] | 21.0 (20.1-22.0) | 20.3 (19.2-21.3) | 21.3 (19.6-23) | 20.4 (18.8-22.0) |
| BCG[h] scar size from revaccination in mm (95% CI)[c] | 8.3 (6.3-10.2) | 7.4 (4.1-10.8) | - | - |
| Current medication use (%) | | | | |
| antihistamine | 0 (0.0) | 1 (6.0) | 0 (0.0) | 0 (0.0) |
| corticosteroid | 0 (0.0) | 0 (0.0) | 1 (6.0) | 0 (0.0) |
| other non-related | 6 (21.0) | 3 (19.0) | 3 (18.0) | 2 (18.0) |
| none | 23 (79.0) | 12 (75.0) | 13 (76.0) | 9 (82.0) |
| Allergy (%)[i] | | | | |
| asthma/wheezing | 6 (21.0) | 2 (12.0) | 4 (24.0) | 2 (18.0) |
| rhinitis/sneezing | 16 (55.0) | 4 (25.0) | 16 (94.0) | 2 (18.0) |
| rhinitis/sneezing combined with asthma/wheezing | 2 (7.0) | 1 (3.0) | 1 (6.0) | 1 (9.0) |
| dermatitis/eczema | 5 (17.0) | 2 (12.0) | 1 (6.0) | 0 (0.0) |
| not allergic | 8 (28.0) | 11 (69.0) | 1 (6.0) | 8 (73.0) |
| n/a | 1 (3.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Allergy symptoms in the past 12 months (%)[i] | 13 (45.0) | 5 (31.0) | 13 (76.0) | 3 (27.0) |
| n/a | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Atopy (%) | | | | |
| to any allergen in the SPT[j] | 17 (59.0) | - | 8 (47.0) | - |
| to Derp[k] in the SPT | 24 (83.0) | - | 14 (82.0) | - |
| SPT conversion 2 months after intervention* | - | 1 (6.0) | - | 1 (9.0) |
| Total IgE (ng/ml)[c] | | | | |
| baseline (T0) | 90.0 (45.4-134.5) | 19.4 (3.2-35.7) | 23.6 (10.8-36.5) | 21.4 (2.2-40.6) |
| two months after intervention (T2) | 66.7 (36.0-97.4) | 21.9 (7.9-36.0) | 34.4 (8.9-60.0) | 25.5 (1.8-49.2) |
| Anti-Derp IgE (kU/ml)[c] | | | | |
| baseline (T0) | 29.40 (13.18-45.63) | 0.00 (0.00-0.00) | 23.70 (6.54-40.85) | 0.00 (0.00-0.00) |
| two months after intervention (T2) | 29.3 (10.5-48.0) | 0.12 (–0.10 to -0.33) | 8.09 (–2.69 to -18.87) | 0.22 (–0.47 to –0.90) |

**95% CI:** 95% confidence intervals; **MW:** minimum wage; **n/a:** information not available; **BMI:** body mass index; **BCG:** Bacille Calmette-Guérin; **ISAAC:** International Study of Asthma and Allergies in Childhood; **SPT**: skin prick test; **Derp:** *Dermatophagoides pteronyssinus*; **IgE:** immunoglobulin E; **T0:** time zero (baseline); **T2:** two months after the intervention. [a]One revaccinated individual not tested. [b]One control individual not tested. [c]Results are expressed as means (95% CI). [d]MW (>5 MW indicates an upper-class or class "A" consumer)[3]. [e]Alcohol use was defined as alcohol consumption at least once per week. [f]Marijuana use. [g]BMI (weight in kg divided by the square of the height in m). [h]BCG. [i]Data obtained from the ISAAC questionnaire. [j]SPT. [k]Derp whole extract. *Percentage of individuals non-reactive to Derp at baseline that became reactive 2 months after revaccination.

enrollment and follow-up flowcharts have been published elsewhere[10]. All individuals had been vaccinated with BCG in early childhood and tested negative for latent tuberculosis (in the tuberculin skin test) and human immunodeficiency virus (HIV) before inclusion in the study. Whole blood cultures (blood diluted 1:10 in Roswell Park Memorial Institute (RPMI) 1640 stimulated with 10μg/ml *Mycobacterium tuberculosis* H37Rv culture lysate) from 46% of BCG-revaccinated individuals in this trial showed consistently higher levels of IFN-γ at 2 and 12 months after the intervention compared with baseline levels and the control group[10]. The fold-increase in the IFN-γ levels at 2 months after revaccination relative to baseline levels (T2/T0) was predictive of the IFN-γ responses measured at 12 months, which exceeded the median values of the control group by at least 2-fold the standard deviation[10].

The clinical trial assessed skin reactivity against respiratory aeroallergens, including *Dermatophagoides pteronyssinus* (Derp), *Blomia tropicalis*, *Blattella germanica*, *Periplaneta americana*, dog epithelium, cat epithelium, pollen mixture IV (*Dactylis glomerata*, *Festuca arundinacea*, *Lolium perenne*, *Phleum pratense*, and *Poa pratensis*), and fungal mixture V [*Aspergillus (A.) Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terrus*, *Penicillium (P.) Penicillium brevicompactum*, *Penicillium expansum*, *Penicillium notatum*, *Penicillium roqueforti*, *Cladosporium fulvum*, and *Cladosporium herbarum*] using skin prick tests [(SPT); ALK-Abelló, São Paulo, Brazil]. Sociodemographic and clinical characteristics were obtained using a standard questionnaire and the International Study of Asthma and Allergies in Childhood (ISAAC)[11] questionnaire. Total serum IgE levels were measured using the Human IgE Flex Set assay and a FACSArray flow cytometer (Becton Dickinson, Palo Alto, CA, USA). Anti-Derp serum IgE levels were measured using an ImmunoCAP kit (ImmunoCAP Phadia AB, Uppsala, Sweden). Multivariate linear models were evaluated, adjusting for a combination of the following variables: gender, age, body mass index (BMI), income, revaccination scar size, occurrence of allergies in the previous 12 months, atopy (defined as a positive SPT and/or anti-Derp IgE level >0.35kU/L at baseline), and the IFN-γ T2/T0 ratio.

The percentage of atopic and non-atopic individuals was not significantly different between the revaccinated and control group (**Table 1**). Demographic characteristics, smoking, drug use habits, and allergy symptoms did not differ between revaccinated and control individuals, either atopic or non-atopic. Atopic individuals were usually sensitized against aeroallergens from the house dust mite *D. pteronyssinus*, as observed in previous studies[12]. Baseline total IgE levels were increased in atopic individuals of the revaccinated group compared with atopic controls. The levels of total and anti-Derp IgE were not significantly different between T2 and T0 in both groups.

Total IgE levels (**Figure 1A** and **Figure 1C**) and anti-Derp IgE levels (**Figure 1B** and **Figure 1D**) remained unchanged up to 12 months after the intervention in the revaccinated (**Figure 1A**and **Figure 1B**) and control (**Figure 1C** and **Figure 1D**) groups. In BCG-revaccinated individuals, total IgE levels at T2 were positively correlated with total IgE levels ($p < 0.0001$; Spearman $r=0.88$) and anti-Derp IgE levels ($p < 0.0001$; Spearman $r=0.54$) at T0, suggesting a lack of IgE modulation from BCG revaccination, which agrees with the results of previous studies[13]. Although the results of bivariate analysis indicated that there was no association between total or anti-Derp IgE levels and the parameters used in the multivariate linear models, these variables were included in the models because of a previously reported correlation with IFN-γ levels upon mycobacterial antigen stimulation after BCG revaccination[9,14] or the relevance of these variables in previous studies investigating determinants of asthma[15].

The modulation of serum IgE (total and anti-Derp-specific) at 2 months after BCG revaccination was evaluated using multivariate linear models (**Figure 1**). Vaccine responsiveness was taken into account by stratifying the BCG-revaccinated group according to the IFN-γ T2/T0 ratio into a high-responder group (IFN-γ T2/T0 ratio >3.262) and a low-responder group (IFN-γ T2/T0 ratio ≤3.262) because this cut-off was predictive of the IFN-γ response to *M. tuberculosis* antigens after 12 months of revaccination, as discussed above[10]. Combinations of the following variables were tested: gender, age, body mass index (BMI), income, revaccination scar size, occurrence of allergies in the previous 12 months, atopy (defined as a positive SPT and/or anti-Derp IgE level >0.35kU/L). The results indicated that these variables did not affect the regression coefficients compared with the bivariate analysis of serum IgE levels (total or anti-Derp) between T2 and T0. Therefore, the results of the two bivariate regression models were analyzed considering the vaccine responsiveness in a stratified analysis (**Figure 1**). There was no significant difference between the regression coefficients of the models describing the relationship between total IgE levels at T2 and T0 in the control and BCG-revaccinated individuals of the low- and high-responder groups (**Figure 1E**). The regression coefficients of the models describing the relationship between anti-Derp IgE levels at T2 and T0 were also close to 1 in all groups (**Figure 1F**), suggesting that BCG did not affect the serum IgE response, regardless of the capacity of responding to the vaccine evaluated by the IFN-γ levels.

The individuals included in the clinical trial reported mild respiratory allergy symptoms, and most had not used medications to treat allergic symptoms in the previous 12 months (**Table 1**). A minority (15%) reported dry cough in the absence of respiratory infection in the previous 12 months; only one individual reported having symptoms after physical exercise that impaired activities of daily living and speech. However, in this study, no clinical evaluations were performed to assess the regression of allergic symptoms after revaccination with BCG. Therefore, we could not determine the possible effects of BCG revaccination on allergic responses in the volunteers of this trial. Notwithstanding, a previous study reported that respiratory function was improved and medication use was reduced after repeated BCG administration in patients with moderate to severe perennial asthma, although the effect on eosinophil counts and sputum cytokine levels was null, and IgE levels were not determined[16].

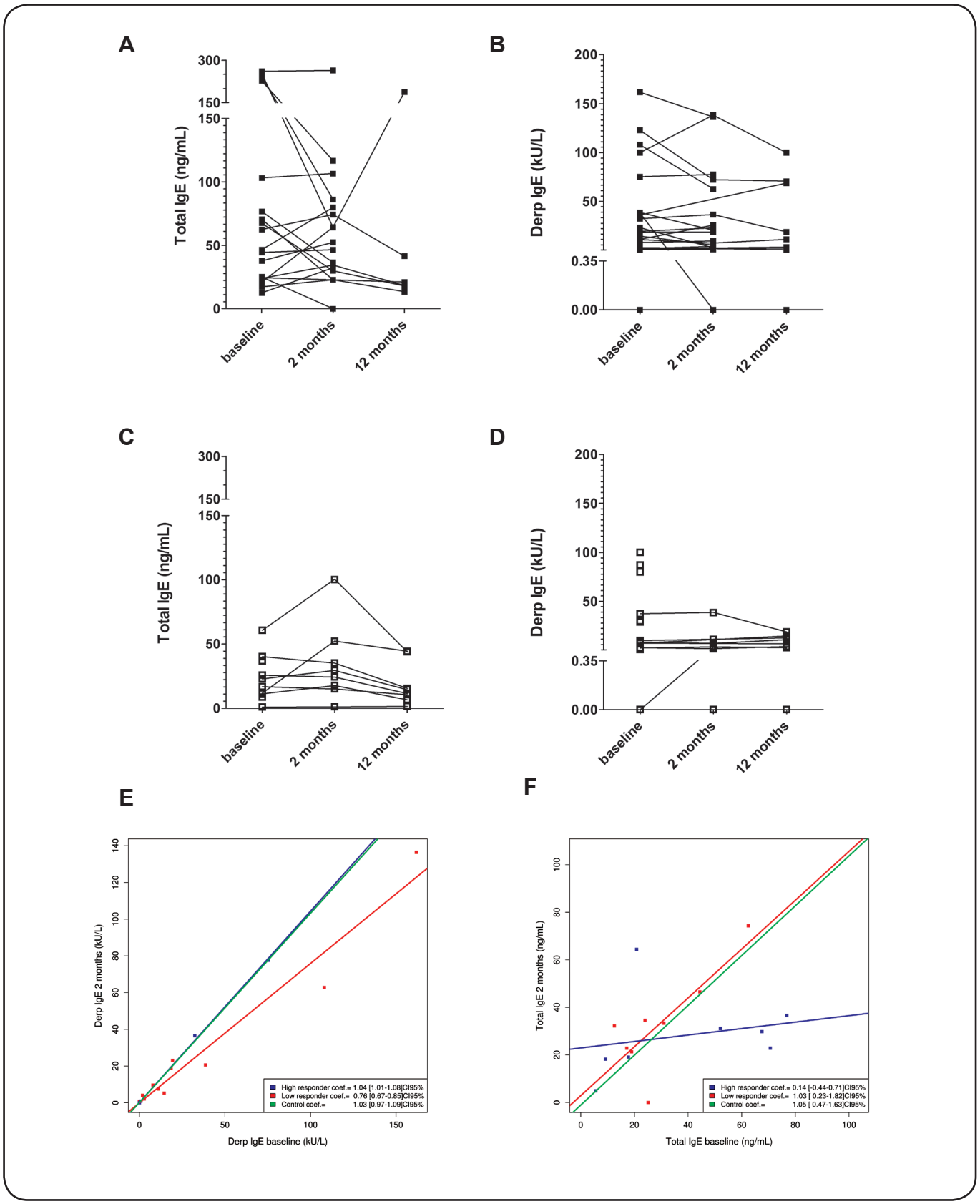


**FIGURE 1: A.** Total serum IgE levels in revaccinated volunteers. **B.** Anti-Derp serum IgE levels in revaccinated volunteers. **C.** Total serum IgE levels in control volunteers. **D.** Anti-Derp serum IgE levels in control volunteers. **E.** Multivariate linear models of the relationship between total serum IgE levels 2 months after baseline quantification and total IgE levels at baseline. **F.** Multivariate linear models depicting the relationship between anti-Derp serum IgE levels 2 months after baseline quantification and total IgE levels at baseline. Confidence intervals at 95% are indicated in brackets. The blue line and squares depict the data from high responders; the red line and squares depict the values obtained for low responders, and the green line and squares represent data from controls. **IgE:** immunoglobulin E; **Derp:** *Dermatophagoides pteronyssinus.*

It has been suggested that the inability of BCG revaccination to modulate atopic responses may be related to an individual's age at the time of antigen exposure and/or the genetic background of the study subjects[2]. In animal models, the effect of the modulation of the Th2 response was protective and improved parameters related to allergic conditions in animals exposed to microbial antigens before allergen sensitization[5]. Children with respiratory allergies who received neonatal BCG in Salvador were protected against asthma[8]. Age at the time of vaccination may have played a protective role in these cases, possibly modulating the children's response before allergic sensitization. It is also possible that the route of administration and antigen load affect the ability of BCG to modulate the atopic response[16,17]. Alternatively, an overlooked effect of non-atopic responses may explain this apparent protection by vaccination with BCG.

In conclusion, BCG revaccination did not modulate total IgE levels in vaccine-responsive individuals and allergen-specific IgE levels in the studied population, regardless of the magnitude of the increase in the IFN-γ levels.

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**Conflicts of interest**

The authors declare that there is no conflict of interest.

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