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Vaccine 21 (2003) 2152–2160

Vaccine

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BCG (Bacille of Calmette–Guérin) revaccination leads to improved in vitro IFN-γ response to mycobacterial antigen independent of tuberculin sensitization in Brazilian school-age children

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Received 30 September 2002; received in revised form 5 November 2002; accepted 12 November 2002

Abstract

Tuberculin skin test (TST) response and cytokine production in finger stab-derived whole blood cultures from 136 BCG scar-positive school-age children were evaluated before and after BCG revaccination. Fifty-four percent of the children increased in vitro production of IFN-γ after revaccination, and this increase was highly significant for previously unresponsive children ($P < 0.0001$). No correlation was found between TST response and cytokine production. Our data suggest that the in vitro IFN-γ response to mycobacterial antigens can be boosted by BCG revaccination and may contribute to the search of correlates of protection to be used for the evaluation of new mycobacterial vaccines.

Keywords: Immunological markers; Tuberculosis: immune response; IFN-γ; TNF-α; IL-10

1. Introduction

Vaccination with the Bacilli of Calmette–Guérin (BCG) has long been applied worldwide to prevent tuberculosis (TB). Protection against tuberculous meningitis has been shown to be consistently high [1]. The varied degrees of BCG protection against pulmonary disease, estimated in several studies [2–4], have contributed to divergences as to the importance of BCG vaccine, and also public health policies for its use [5–7]. Nevertheless, BCG vaccination seems to be effective against TB in several countries, and additionally may provide significant protection against other diseases [8–10].

Substitute vaccines against TB have been proposed [11]. However, expensive trials with large numbers of tested individuals will be necessary to estimate their efficacy, given the low incidence and the long duration of asymptomatic infection in *Mycobacterium tuberculosis*-bearing individuals [12]. Cost and ethical considerations regarding the substitution of the well-known BCG vaccine in a vaccine trial in large human populations at risk for tuberculosis, a disease requiring long treatment duration, with potential side effects, constitute further constraints in new TB vaccine development.

The identification of markers that would correlate with protection against the disease may facilitate the evaluation of new candidate TB vaccines. The present knowledge on the immune mechanisms involved in mycobacterial killing and control of infection has lead to suggestions for candidate immunological surrogates of protection [12]. Although not yet shown to correlate with protection, IFN-γ production in peripheral blood cultures stimulated with mycobacterial antigens, induction of memory-type/antigen specific T cells, or the capacity to kill intracellular mycobacteria upon in vitro

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0264-410X/02/$ – see front matter
PII: S0264-410X(02)00721-1

infection have been shown to be augmented in tuberculosis patients that recover from disease after treatment completion [13], and in BCG vaccinated individuals [14]. Assuming an important role of memory-type responses in the control of mycobacterial infection, investigation of immunological parameters associated with revaccination might contribute to identify potential immunological correlates of protection.

BCG vaccination for children under 1 year has been recommended in Brazil since 1976, which is usually administered to neonates [15]. The few studies available point to a protective effect of BCG neonatal vaccination against tuberculous meningitis [16–18] and leprosy [19–21] in Brazil. Based on these findings and on studies that indicate a decline in the immune response conferred by BCG vaccination with age [22–24], the Brazilian Ministry of Health presently recommends re-vaccinating Brazilian school-age children [15,25]. In 1996, a large study was initiated to estimate BCG revaccination efficacy against all forms of the disease, which is currently in progress [26]. In the context of this study, we addressed the pattern of candidate markers of protection against mycobacteria after revaccination with BCG of Brazilian school-age children. Our evaluations included tuberculin skin test (TST) and in vitro measures of the cytokines IFN-γ, TNF-α and IL-10 upon stimulation of whole blood cultures with *Mycobacterium tuberculosis* crude antigen, as production levels of these cytokines have been associated with protection and/or disease severity [27]. We show that IFN-γ in vitro production before BCG revaccination is associated with younger age, and that children with initial poor IFN-γ in vitro production to mycobacterial antigen develop a significant increase of IFN-γ production after BCG revaccination. These findings lend further support to a possible use of IFN-γ response as a putative 'best immunological correlate of protection', in agreement with present models of protective immunity against mycobacterial diseases.

2. Methods

2.1. Blood collection, tuberculin test and BCG revaccination

This study was performed in the context of an ongoing trial to estimate the efficacy of a second dose of BCG at school-age [26]. The population evaluated consisted of children aged 7–15 years attending one state school in the urban area of Salvador (Bahia, Brazil), that was selected for BCG revaccination. Parents and children reserved the right to refuse participation in the study, both on the day of BCG revaccination and 8 weeks post-BCG revaccination, when blood samples were collected for analysis. All consenting children with previous BCG vaccination assumed by the presence of one suggestive BCG scar on day 0 were evaluated. BCG scar has been shown to be a good indicator of previous BCG vaccination in this population [28]. Personal data from all participant children were recorded from the school's registry using a standardized form. All children were apparently healthy at both sample collection points.

On day 0, children were submitted to finger prick for blood collection, performed after local disinfection with 70% ethanol, using a disposable sterile lancet (Inlab, Sao Paulo, Brazil). This procedure yielded approximately 50 μl of blood, which was collected in a heparinized capillary tube for micro-hematocrit determination (75 × 1 mm, Perfecta Ind. Com. de Laminas de Vidro LTDA, Sao Paulo, Brazil). Then, the tuberculin test was performed by intradermal injection of 0.1 ml of reagent (PPD RT SSI 23, Statens Serum Institut, Denmark) on the volar surface of the right arm, with a 27G sterile needle. Finally, children were given BCG revaccination (Moreau strain, FIOCRUZ, Rio de Janeiro, Brazil). Forty-eight hours after intradermal injection, the PPD reactivity was read by two qualified nurses trained according to the procedures from the Tuberculosis Control Program of the Brazilian Ministry of Health. Children with PPD readings below 5 mm were considered non-reactors; children with PPD readings ranging from 5 to 10 mm were considered weak reactors and those with PPD readings of 10 mm or above were classified as strong reactors.

Eight weeks after BCG revaccination the children were re-submitted to finger prick blood collection and tuberculin test, by the same nurses, following the procedures described above.

2.2. Whole blood cultures

Blood samples were diluted 1:10 in RPMI medium (GibcoBRL, Rockville, MD) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (GibcoBRL). Two hundred microliters of diluted blood sample were added to each of two wells of a flat-bottom 96 well plate (Corning, NY). One well was stimulated with *M. tuberculosis* H37Rv culture supernatant filtrate (*M. tuberculosis* CSF, 10 μg/ml) and one was left untreated. Plates were incubated in humidified atmosphere (37 °C, 5% CO_2) for 3 days, after which culture supernatants were recovered and kept at −20 °C until analysis. The method of whole blood culture for cytokine evaluation has been explored in detail [29].

2.3. Cytokine detection in culture supernatants

IFN-γ and TNF-α levels in the supernatants from whole blood cultures performed on day 0 and 8 weeks after BCG revaccination were measured by enzyme-linked immunosorbent assay (ELISA), using Duo-Set (Genzyme, Cambridge, MA).[1] IL-10 levels were measured using the kit Quantikine (R&D Systems, Minneapolis, MN). Cytokine evaluation

[1] From 136 pre-BCG revaccination cultures, 134 were evaluated for TNF-α levels, and 122 were evaluated for IFN-γ levels. One hundred thirty-one cultures from post-BCG re-vaccination whole blood samples were evaluated for both IFN-γ and TNF-α, and one was evaluated only for IFN-γ. Due to technical difficulties, we were only able to assess IL-10 production in 42 post-BCG revaccination whole blood cultures.

Table 1
Population and immunological parameters in school-age children before BCG revaccination

	n[a]	PPD (mm)[b]	Age (years)	Sex ratio (M:F)[a]	IFN-γ (pg/ml)[b]	TNF-α (pg/ml)[b]
Non-reactors (PPD <5 mm)	126	0 (0–4)	11 (7–15)	52:74	47 (0–678)	327 (0–2169)
Weak reactors (5 < PPD <10 mm)	9	7.5 (5–9)	10 (7–13)	4:5	81 (0–389)	371 (64–744)
Strong reactors (PPD >10 mm)	1	14	11	1:0	106	2183.7
Total	136	0 (0–14)	11 (7–15)	57:79	53 (0–678)	336 (0–2184)

[a] Number of children classified in the group.
[b] Median (min–max).

sensitivity in our experiments was 15.6 pg/ml for IFN-γ and TNF-α, and 15 pg/ml for IL-10.[2] Mean IFN-γ levels for non-stimulated wells were not significantly different from the detection limit ($P > 0.05$, Wilcoxon signed rank test). Mean TNF-α levels for non-stimulated wells reached 192.1 pg/ml (S.D., 250.7 pg/ml). TNF-α levels above detection limit for non-stimulated samples are in agreement with previous reports for whole-blood cultures performed with blood collected by finger-stab [30]. IL-10 levels were not measured in these samples. All results mentioned in this report refer to cytokine levels in *M. tuberculosis* CSF-stimulated wells.

2.4. Data analyses

Analyses were performed using Corel Quattro Pro (Corel Co. And Corel Co. LTD, Ontario, Canada) and GraphPad Prism v 3.00 (GraphPad Inc., San Diego, CA). Comparisons between age groups and low IFN-γ (lo IFN) versus high IFN-γ (hi IFN) groups were done using the Mann–Whitney test. Comparisons between pre-BCG revaccination versus post-BCG revaccination values were done using the Wilcoxon signed rank test. Tuberculin skin test non-converter, weak converter and strong converter groups were compared using the Kruskal–Wallis test. Spearman's correlation coefficients were calculated to assess degree of association between the variables studied.

3. Results

3.1. BCG-vaccinated population

Of 694 children contacted, enrolled in the same public school in the urban area of Salvador (Bahia, Brazil), 370 (53% of the contacted children) did not receive BCG vaccination and 220 (68% of the vaccinated children) were not included in the study following their own refusal or absence, or their parents disapproval at any blood sample collection point. Thirteen consenting children did not have a scar suggestive of previous BCG vaccination and one had incomplete registry, and were therefore excluded from our analysis. One hundred thirty-six BCG scar-positive children with tuberculin skin test and blood collection on day 0 and 8 weeks post-BCG revaccination were evaluated in this study. The children evaluated were 7–15 years old, with 57 boys, and 79 girls (Table 1). No significant differences were found by gender for the parameters evaluated, except for TNF-α levels in post-BCG revaccination cultures. Surprisingly, girls responded with significantly higher post-BCG revaccination TNF-α production than boys (median of 308.9 (min = 0.0–max = 1102.0) versus 211.3 (0.0–838.2) for boys, $P = 0.007$, Mann–Whitney test).

3.2. Immunological evaluations prior to BCG revaccination

In pre-BCG revaccination whole blood cultures, IFN-γ levels correlated negatively with age ($P = 0.02$, Spearman $r^2 = -0.21$). Cultures from children with age below median responded more frequently to antigen challenge in terms of IFN-γ production than cultures from elder children ($P = 0.02$, Fig. 1). In cultures with IFN-γ levels above the median (52.9 pg/ml, Table 1), the production of this cytokine was positively correlated to TNF-α levels, as shown in Fig. 2 ($P = 0.008$, Spearman $r^2 = 0.34$).

However, most children did not respond to the tuberculin test prior to BCG revaccination (Table 1). Nine children were weak reactors, and only one child was a strong reactor. No cytokine evaluated in this study correlated with skin reactivity against tuberculin ($P > 0.05$, Spearman correlation analyses). There was no statistically significant difference between tuberculin reactive and tuberculin non-reactive children in terms of age or the production of IFN-γ or TNF-α upon in vitro stimulation with *M. tuberculosis* CSF ($P > 0.05$, Mann–Whitney test).

3.3. Immunological evaluations post-BCG revaccination

Eight weeks after BCG revaccination, 78% of tuberculin non-reactive children converted to a positive PPD test (Table 2). Children classified as PPD non-reactors prior to BCG revaccination (Table 1) were subdivided in three categories: children that remained non-reactors after vaccination

[2] In every ELISA plate evaluated, four wells with the supernatant of whole blood culture stimulated with concanavalin A (Sigma) were used as an internal positive control. IFN-γ mean production levels in concanavalin A positive control wells attained 724.8 pg/ml (standard deviation, 67.54 pg/ml) in pre-BCG revaccination evaluations, and 757.2 pg/ml (S.D., 34.03 pg/ml) in post-BCG revaccination measurements.

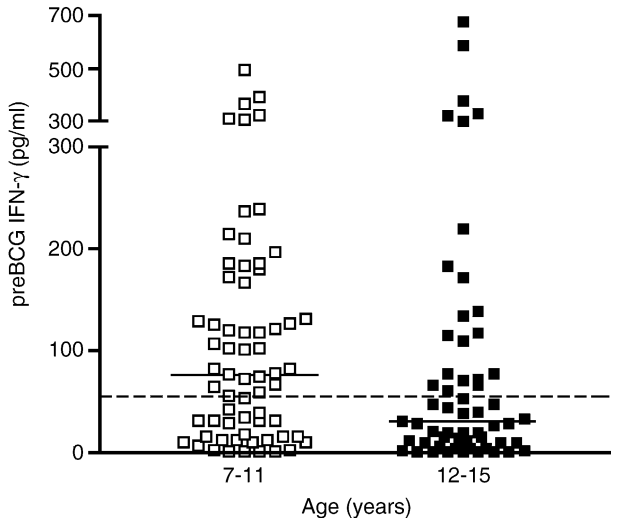

Fig. 1. Pre-BCG revaccination IFN-γ levels in finger stab-derived whole blood cultures stimulated with *M. tuberculosis* CSF, according to age. Open squares, levels in cultures from children up to 11 years old; closed squares, levels in cultures from children 12–15 years old. Difference is significant by the Mann–Whitney test ($P = 0.02$). Horizontal bar represents the median for the group. Dashed line represents the limit of sensitivity for cytokine detection by ELISA.

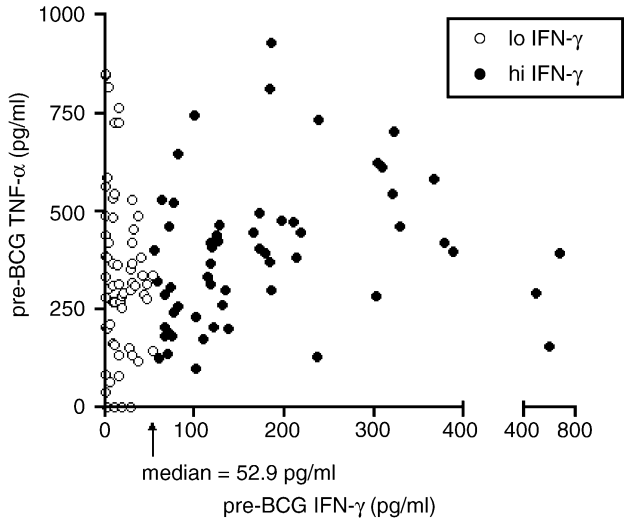

Fig. 2. Correlation between IFN-γ and TNF-α production in pre-BCG revaccination finger stab-derived whole blood cultures. Open circles, levels in cultures from children with IFN-γ production below the median (52.9 pg/ml)—"lo IFN-γ". Closed circles, levels in cultures from children with IFN-γ production above the median—"hi IFN-γ". Correlation is significant in the hi IFN-γ group ($P = 0.008$, $r^2 = 0.34$; Spearman correlation analysis).

(non-converters), children that changed to a skin test response diameter between 5 and 10 mm (weak converters) and children that converted to a skin test response diameter higher than 10 mm (strong converters). There was no difference in post-vaccination IFN-γ, TNF-α or IL-10 in vitro production among non-converters, weak converters or strong converters ($P > 0.05$, Kruskal–Wallis one-way ANOVA). No correlations were found between PPD reactivity and in vitro cytokine responses at 8 weeks after BCG revaccination ($P > 0.05$, Spearman correlation analysis).

Given the central role that IFN-γ plays in the immune response against mycobacterial infection, we subdivided the evaluated children in two groups, according to their in vitro capacity to produce this cytokine in response to stimulation with *M. tuberculosis* CSF prior to revaccination. This strategy was used to verify whether BCG revaccination had improved the IFN-γ response to in vitro challenge with *M. tuberculosis* CSF antigen, and for which group revaccination had been most beneficial. The "lo IFN-γ" group was composed of children with pre-BCG revaccination IFN-γ levels below the population median (52.9 pg/ml, Table 1), and the "hi IFN-γ" group consisted of children with IFN-γ levels above this median.

After BCG revaccination, 72% (45 of 62) children classified in the lo IFN-γ group mounted increased IFN-γ production upon in vitro challenge with *M. tuberculosis* CSF, leading to a significant improvement in IFN-γ response (Fig. 3A). Median IFN-γ production before revaccination increased from near detection limit (12.2 pg/ml) to 46.2 pg/ml post-BCG revaccination levels ($P < 0.0001$, Wilcoxon signed rank test). In contrast, only 35% of the children classified in the hi IFN-γ group (21 out of 60) showed increased in vitro production to *M. tuberculosis* CSF after BCG revaccination. The remaining have either

Table 2
Population and immunological parameters in school-age children 60 days after BCG revaccination

	n[a]	PPD (mm)[b]	Age (years)	Sex ratio (M:F)	IFN-γ (pg/ml)[b]	TNF-α (pg/ml)[b]	IL-10 (pg/ml)[b]
Prevaccination PPD reactors[c]	10	13 (2–15)	10 (7–13)	5:5	46 (0–553)	276 (0–557)	125 (107–410)
Prevaccination PPD non-reactors[d]:							
Non-converters (PPD <5 mm)	28	2 (0–4)	12 (7–14)	16:12	51 (0–608)	219 (0–838)	140 (30–947)
Weak converters (5 < PPD <10 mm)	52	7 (5–9)	12 (8–15)	17:35	58 (0–642)	297 (0–1022)	157 (0–516)
Strong converters (PPD >10 mm)	46	12 (10–17)	11 (7–14)	19:27	45 (0–793)	238 (0–909)	148 (0–497)
Total	136	8 (0–17)	11 (7–15)	57:79	54 (0–793)	265 (0–1022)	144 (0–947)

[a] Number of children classified in the group.
[b] Median (min–max).
[c] Pre-vaccination PPD >5 mm (see Table 1).
[d] Pre-vaccination PPD <5 mm.

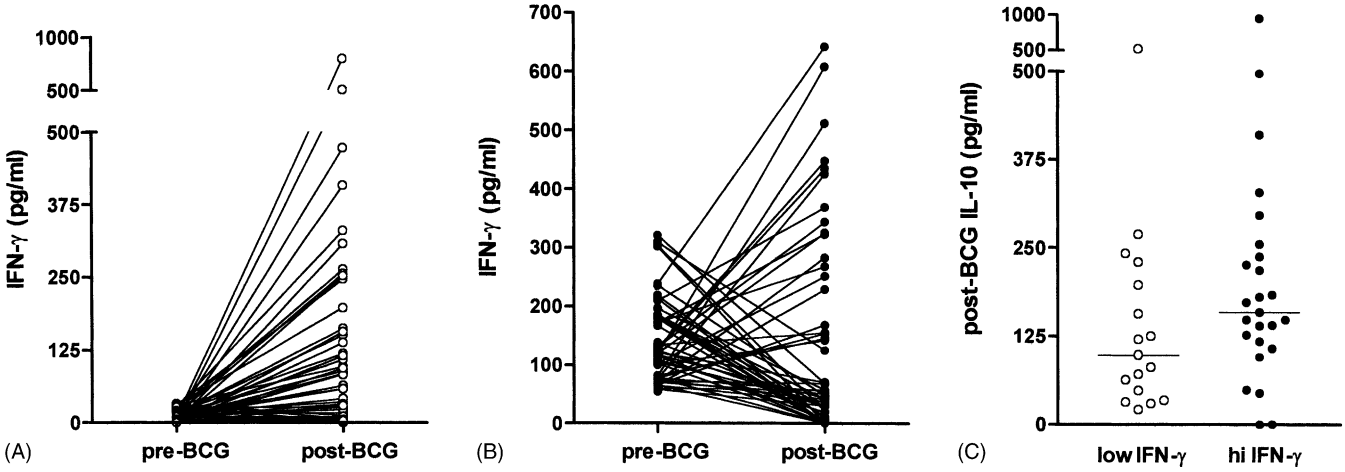

Fig. 3. Evolution of cytokine levels in finger stab-derived whole blood cultures from pre-BCG revaccination high "hi IFN-γ" vs. low IFN-γ "lo IFN-γ" producers. (A): pre-BCG revaccination vs. post-BCG revaccination IFN-γ levels in the lo IFN-γ group prior to BCG revaccination. Difference is significant by the Wilcoxon signed rank test ($P < 0.0001$); (B): pre-BCG revaccination vs. post-BCG revaccination IFN-γ levels in the hi IFN-γ group prior to BCG revaccination. Difference is not significant by the Wilcoxon signed rank test ($P > 0.05$); (C): post-BCG revaccination IL-10 levels in pre-BCG revaccination high vs. low IFN-γ producers. Horizontal bar represents the median for the group. Difference is not significant by the Mann–Whitney test ($P > 0.05$).

maintained similar pre-vaccination levels or decreased capacity to produce IFN-γ upon in vitro stimulation ($P > 0.05$, Wilcoxon signed rank test, Fig. 3B). However, the increase in IFN-γ production was not significant when comparing pre- and post-BCG revaccination levels among younger children (age group of 7–11 years old children) or older children (age group of 12–15 years old children) ($P > 0.05$, Wilcoxon signed rank test, Fig. 4).

The observed increase in IFN-γ in vitro levels by previously unresponsive children was not associated with TNF-α or IL-10 production. Post-BCG revaccination IFN-γ response did not correlate with TNF-α production; neither for the whole population studied, nor considering lo IFN-γ versus hi IFN-γ groups separately ($P > 0.05$, Spearman correlation analysis). There was no significant difference between lo IFN-γ versus hi IFN-γ children when comparing post-BCG revaccination IL-10 levels ($P > 0.05$, Mann–Whitney test, Fig. 3C). TNF-α levels presented a significant positive correlation with IL-10 levels in post-BCG revaccination cultures ($P = 0.003$, Spearman $r^2 = 0.53$; Fig. 5). TNF-α levels diminished significantly only in post-BCG revaccination cultures from boys ($P < 0.0001$, Wilcoxon signed rank test, Fig. 6), but there was no difference in IL-10 levels between boys and girls ($P > 0.05$, Mann–Whitney test). Median TNF-α production in boys decreased from 353.3 pg/ml before revaccination to 211.3 pg/ml post-BCG revaccination levels.

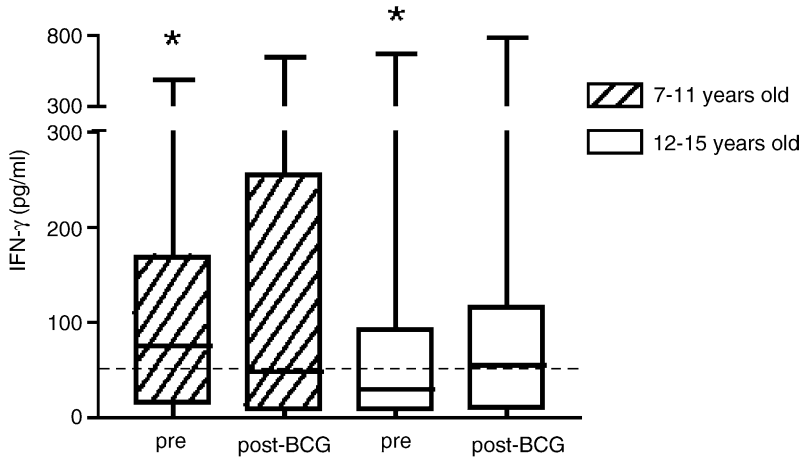

Fig. 4. Evolution of IFN-γ levels in finger stab-derived whole blood cultures from BCG re-vaccinated children stratified by age. Boxes represent values between the 25th and the 75th quartiles; middle horizontal bar stands for the median level in the group. Error bars indicate maximum and minimal values observed. Hatched boxes, 7–11 years old children; clear boxes, 12–15 years old children. (*) Difference between pre-BCG revaccination IFN-γ levels of 7–11 years old vs. 12–15 years old children is significant by the Mann–Whitney test ($P = 0.02$).

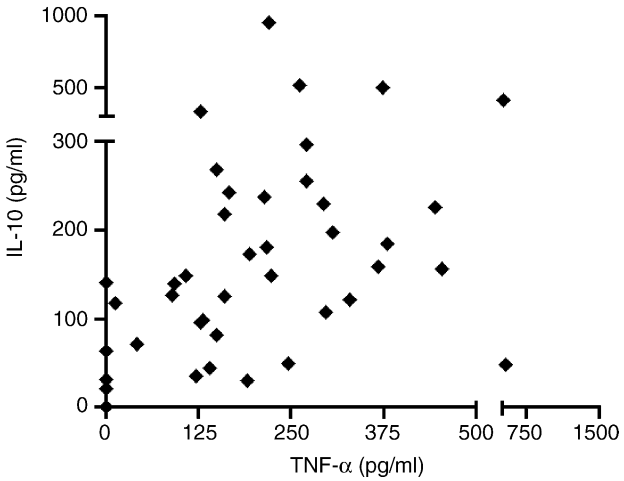

Fig. 5. Correlation between TNF-α and IL-10 levels in post-BCG revaccination finger stab-derived whole blood cultures. Correlation is significant by the Spearman correlation analysis ($P = 0.003$; $r^2 = 0.53$).

4. Discussion

Tuberculosis is considered a global epidemic, and major efforts are under way to design new vaccines that may consistently induce protection against TB worldwide [11]. The evaluation of vaccine protection is complicated by the lack of animal models that accurately mimic human TB, and by the lengthy, low rate of disease development [12]. In the present study of the immune response boosting in BCG revaccinated populations, we contribute to furthering the search for potential surrogate markers of protection for pre-selection of TB candidate vaccines.

PPD reactivity is currently regarded both as a marker for correct BCG administration [31] as well as to diagnose TB infection [32]. PPD reactivity induced by BCG vaccination/revaccination is regarded as a confounding factor in early TB control [33]. In a study analyzing BCG-vaccinated volunteers in Sweden, high IFN-γ levels were associated to tuberculin skin tests above 15 mm [34]. Another study evaluating PPD reactivity in BCG vaccinated children suggests that tuberculin skin reaction diameters above 16 mm should be considered indicative of TB infection [35]. In our study population, only one child presented a skin test reaction diameter higher than 10 mm, and nine children considered PPD reactive had tuberculin reactions between 5 and 10 mm. We suggest therefore that the immune responses assessed in our study population are likely to result from earlier BCG vaccination, rather than from a latent TB infection. In our study, PPD reactivity did not correlate with in vitro IFN-γ production, contrary to the findings from previous studies with non-BCG vaccinated individuals in Malawi and England [36,37]. The authors associated in vitro IFN-γ production upon stimulation with *M. tuberculosis* antigens in these populations with exposure to environmental mycobacteria.

Increased IFN-γ response upon in vitro challenge with mycobacterial antigen is presently considered "imperfect but the best presently available correlate of protection" against TB [12]. We show that detectable pre-BCG revaccination IFN-γ levels above threshold were found in whole blood cultures from approximately 70% of the children evaluated, and were more common in cultures from children with age below the median (11 years). In these pre-BCG revaccination IFN-γ-positive cultures, IFN-γ and TNF-α in vitro levels presented a positive correlation, which may well indicate the capacity of mounting a Th1-type response against mycobacteria in IFN-γ-producing individuals.

Previous studies have documented the decline of PPD response with age in BCG vaccinated populations [38]. A concurrent decline of specific immunity conferred by BCG vaccination has also been documented [39]. We have demonstrated a persistent IFN-γ response in the absence of PPD reactivity, especially in children 7–11 years old. Moreover,

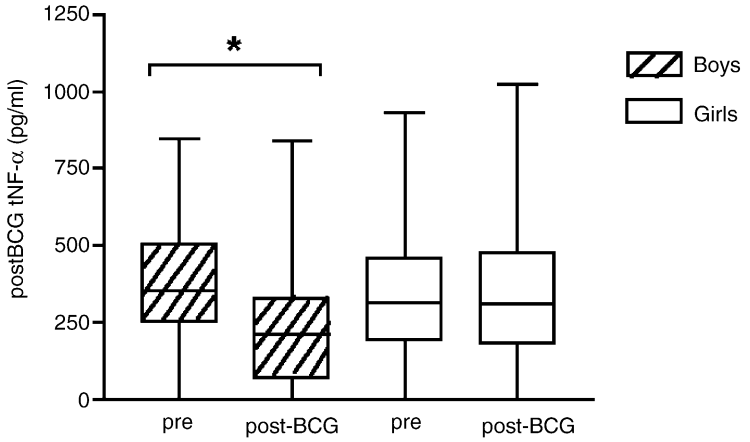

Fig. 6. Evolution of TNF-α levels in finger stab-derived whole blood cultures from BCG re-vaccinated children stratified by sex. Boxes represent values between the 25th and the 75th quartiles; middle horizontal bar stands for the median level in the group. Error bars indicate maximum and minimal values observed. Hatched boxes, boys; clear boxes, girls. (∗) Difference is significant by the Wilcoxon signed rank test ($P < 0.0001$). The limit of sensitivity for cytokine detection by ELISA is 15.6 pg/ml.

given that more than half of the children evaluated presented an IFN-γ production above threshold, the decline of IFN-γ production with time since vaccination may be less abrupt than the estimates reported for PPD responsiveness. These results are also in agreement with the notion that PPD reaction induced after BCG vaccination may not be indicative of the degree of protection against TB [40]. We observed, however, a high variability in IFN-γ production among the children evaluated, even in those between 7 and 11 years of age. In spite of being mandatory in Brazil since the late 70's, BCG vaccination coverage estimates for children under 1 year of age in the study area were less than 80% before 1990 [41]. Given these estimates, we cannot rule out that some of the children that presented high in vitro IFN-γ production prior to BCG revaccination may have been vaccinated later in life.

After BCG revaccination, most of the children converted to a positive tuberculin skin test, but no correlations were found between tuberculin reaction diameter or skin test conversion and cytokine production upon in vitro antigen challenge post-BCG revaccination. Black and co-workers [37] have compared the IFN-γ in vitro response against mycobacterial antigens and skin test responsiveness before and after BCG vaccination of 12–14 year old British children and 10–28 years old volunteers in Malawi. In England, where BCG efficacy against pulmonary tuberculosis is high [3], the association between IFN-γ response and PPD reaction diameters is still present after BCG prime vaccination, while in Malawi, where BCG confers no protection [3], this association becomes weaker after vaccine administration. Our results in the Brazilian population are therefore in agreement with the reported dissociation between IFN-γ and TST responses, and may be influenced by a booster effect of revaccination. The exact significance of these findings in the context of mechanisms of protection is still unclear. We cannot rule out that tuberculin sensitization at the first evaluation may have contributed significantly to the observed increase in skin test responsiveness 8 weeks after BCG revaccination in our study population, as previously shown [42].

Comparing BCG-vaccinated volunteers from England and India, Cheng and co-workers have failed to demonstrate an association between IFN-γ or TNF-α in vitro activity 8 weeks post-BCG vaccination and protection against TB [14]. We demonstrated a decrease in the TNF-α response to mycobacterial antigens, but surprisingly it was restricted to the boys studied. TNF-α is considered the main cytokine involved in chemokine induction and formation of the granulomatous response against the bacillus [27]. On the other hand, diminished TNF-α production was shown to have a beneficial effect in the control of TB infection [43,44] and may be particularly important to prevent severe pulmonary disease [45]. The biological significance of the sex difference observed remains to be investigated. BCG revaccination has been shown to improve the protection against leprosy [8], and increased in vitro IFN-γ response to *M. leprae* crude antigen associated with lower TNF/IL-10 ratio has been demonstrated in exposed leprosy contacts without disease upon BCG revaccination [46]. In our study, it was not possible to evaluate the changes in IL-10 production upon BCG revaccination of the participating children. However, we have found a positive correlation between post-BCG revaccination TNF-α levels and IL-10 production, which would be in agreement with a possible role for IL-10 in the modulation of TNF-α response [47].

BCG revaccination was able to increase IFN-γ production in whole blood cultures of children with low IFN-γ in vitro levels prior to BCG revaccination. First BCG vaccination was recently shown to increase IFN-γ production in individuals with low baseline IFN-γ response [37]. BCG-vaccinated individuals have been shown to mount an efficient in vitro response to challenge with viable bacilli or mycobacterial antigens [48–50]. Even studies with vaccinated individuals from areas where BCG has apparent null efficacy against TB demonstrate a Th1-type immune response with strong cytotoxic activity against the bacilli in vitro [14]. It has been suggested that in such populations BCG cannot boost protection further than that conferred by previous exposure to environmental mycobacteria, as seen in animal models [51,52]. On the other hand, current estimates of BCG protection against mycobacterial disease have been criticized for not taking into account the decrease in disease severity upon BCG revaccination [53], which may undervalue its importance in TB control. The design of a better vaccine against TB may actually imply the improvement or overpowering of the observed effects of BCG on the immune response to mycobacterial antigens. Whether our findings are in agreement with present models of protective immunity against mycobacterial diseases will be clearer in the light of the results of the ongoing trial to estimate efficacy of BCG revaccination in our population.

Acknowledgements

To Dr. Johan Van Weyenbergh and Dr. Ana L. Bierrenbach for helpful comments, to Dr. Lee W. Riley for kindly reviewing the manuscript and to Mr. Jorge Tolentino, Mrs. Raimunda dos Santos and Mrs. Rosangela Fontes for technical help. This work was supported by CNPq (Brazilian National Council for Scientific and Technological Development), grant no. 521171/1998–9; Brazilian Ministry of Health (MS); and Oswaldo Cruz Foundation (FIOCRUZ). The study was undertaken in the population of a trial funded by National Health Foundation (FUNASA) (Brazil) and Department for International Development (UK). TB, BDF and LPC received fellowships from CNPq.

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