

Original article

Effects of BCG revaccination on asthma

In a study conducted 1 year ago, we found that Th1 immune enhancement following Bacille Calmette–Guérin (BCG) vaccination effectively suppressed human asthma. To investigate whether revaccination would further improve lung function, BCG vaccine was given again. Current lung function tended to improve in the Repeated BCG group ($n = 9$), but not in the Single BCG group (previously the placebo group) ($n = 11$), compared with that 1 year ago. The BCG vaccination improved lung function in both groups, and the Repeated BCG group showed a significant increase in the peripheral blood interferon γ /interleukin 4 ratio. These findings suggest that repeated BCG vaccinations might be effective in asthma therapy.

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As Th1 and Th2 cells are reciprocally regulated, several animal studies on Th2-associated diseases including asthma have shown to reverse the Th2 response into a protective Th1 response. One year ago, we had performed a randomized controlled trial of Bacille Calmette–Guérin (BCG) vaccination in human asthma, showing a significant increase in pulmonary function and a significant reduction in the need for asthma medication (1). However, despite the clinical benefit seen, BCG vaccination appeared to have no apparent suppressive effect on the Th2 immune response. As it is well known that the so-called 'secondary immune response' mediated by memory cells is more effective than the primary response, we tested whether the strong Th1 immune response induced by BCG revaccination would clearly antagonize the Th2 response and further improve lung function in asthmatics.

Material and methods

Twenty of 43 patients with moderate-to-severe asthma who had participated in the previous study (1) (previously the BCG group: $n = 9$; previously the placebo group: $n = 11$) participated in and completed this follow-up study (May–November, 2001). It was not easy for us to get subjects' cooperation repeatedly and only those who wanted to participate in the present study were enrolled. The ethical review board of Chonnam University Hospital, Gwangju, Korea approved this study and all patients provided written informed consent.

All subjects received percutaneous injection of 58.2×10^7 CFUs BCG vaccine (Tokyo-172 strain; Japan BCG laboratory, Tokyo, Japan) on the first day of a 12-week treatment period. Spirometric measurements were performed before treatment and at weeks 4, 8 and 12 following vaccination.

Adequate sputum samples could be obtained in only 28 of 43 patients in the previous study (1). Therefore, measurement of cytokines was carried out using blood samples instead of sputum taken before treatment and 12 weeks after vaccination in this study. Fresh peripheral blood mononuclear cells (PBMC) were separated by Hypaque–Ficoll density-gradient centrifugation and cultured at a concentration of 1.5×10^6 cells/ml in the presence of 50 ng/ml phorbol myristate acetate (Sigma Chemical Co., St Louis, MO) and 1 μ g/ml ionomycin (Sigma) for 48 h. Supernatants were collected and stored at -20°C until further analysis. Concentrations of interleukin (IL)-4 and interferon (IFN)- γ were measured using commercially available enzyme linked immunosorbent assay kits (IL-4: Quantikine HS, R & D Systems, MN; IFN- γ : Endogen, Woburn, MA). The minimum detectable concentration is 0.13 pg/ml for IL-4 and 2 pg/ml for IFN- γ .

For statistical analysis, categorical variables were compared using Fisher's exact test for intergroup differences. Continuous variables were analyzed with the unpaired Student's *t*-test for intergroup difference and the paired Student's *t*-test for intragroup difference. A $P < 0.05$ value was considered statistically significant.

Results

The characteristics of the two groups were similar, except age (repeated BCG vs single BCG: 41.2 ± 2.6 vs 54.2 ± 2.7 years, $P < 0.01$, Table 1). All patients in this study showed evidence of past exposure to mycobacteria. The mean time interval between BCG vaccinations in the present study and BCG or placebo vaccinations in the previous study was 12.5 months.

In the Repeated BCG group, the present baseline values of FEV₁ tended to be higher than in the past, although not statistically significant (2.25 ± 0.24 l vs

Table 1. Baseline characteristics

	Single BCG (n = 11)	Repeated BCG (n = 9)
Age (years)	54.2 ± 2.7	41.2 ± 2.6*
Sex (Male/Female)	5/6	3/6
Duration of asthma (years)	10.7 ± 3.3	14.4 ± 2.3
Atopy on skin prick test (%)	8 (73)	7 (78)
Rhinitis (%)	2 (18)	4 (44)
Evidence of past exposure to mycobacteria (%)		
Old healed tuberculosis lesion on chest radiographs	6 (55)	2 (22)
History of antituberculosis chemotherapy	3 (27)	0 (0)
Previous childhood BCG vaccination scar	3 (27)	7 (78)
Positive tuberculin test (≥5 mm skin induration)	9 (82)	8 (89)
Any one of the above four characteristics	11 (100)	9 (100)
BCG vaccination in our previous study (1)	0 (0)	9 (100)
FVC (% pred.)	87.8 ± 2.8	93.1 ± 3.6
FEV ₁ (% pred.)	73.6 ± 1.9	78.9 ± 3.8
Regular medications (%)		
Theophylline	10 (91)	8 (89)
Oral β ₂ -agonist	1 (9)	0 (0)
Leukotriene receptor antagonist	9 (82)	7 (78)
Inhaled long-acting β ₂ -agonist	10 (91)	8 (89)
Inhaled corticosteroid	10 (91)	9 (100)

Data are mean ± SEM. FVC, forced vital capacity; FEV₁, forced expiratory volume in 1 s; BCG, Bacille Calmette–Guérin. **P* < 0.01 vs single BCG vaccine. Statistical analyses were performed using the unpaired Student's *t*-test for continuous variables and Fisher's exact test for categorical variables.

2.40 ± 0.16 l, mean ± SEM; *P* = 0.36). In contrast, the values were not different from the past in the Single BCG group. BCG vaccinations significantly increased FEV₁ in both groups. Mean FEV₁ values at weeks 0, 4, 8

and 12 were 2.40 ± 0.16, 2.66 ± 0.17, 2.56 ± 0.16 and 2.55 ± 0.15 l in the repeated BCG group and 2.14 ± 0.11, 2.38 ± 0.14, 2.35 ± 0.14, and 2.33 ± 0.13 l in the single BCG group (all *P* < 0.05; Fig. 1).

The actual values of both IFN-γ and IL-4 were progressively decreased following BCG vaccination in both groups. However, the IFN-γ/IL-4 ratio was significantly increased from 117.1 ± 21.9 to 236.8 ± 65.4 at weeks 4 (*P* < 0.05) and to 268.2 ± 67.9 at week 8 (*P* < 0.05) following the BCG revaccination, while not following the single BCG vaccination (Table 2). When data from subjects aged < 60 years in single BCG group (41.2 ± 2.6 vs 47.8 ± 2.8 years, *P* > 0.05) were analyzed to exclude any differential effect of age on IFN-γ/IL-4 ratio, there were no alterations in the result.

There were no systemic adverse reactions after the BCG revaccinations. Erythema at the site of vaccination occurred in all subjects. One (9%) of the single BCG group and two (22%) of the repeated BCG group reported soreness at the site. The skin reactions in all subjects faded over 12 weeks.

Discussion

In this study, BCG revaccination further improved lung function and resulted in an apparent increase in the IFN-γ/IL-4 ratio, shifting the Th1/Th2 balance toward Th1, confirming the results of our previous study (1). This beneficial effect of BCG is corroborated by an investigation by Arkwright & David (2), which showed that an

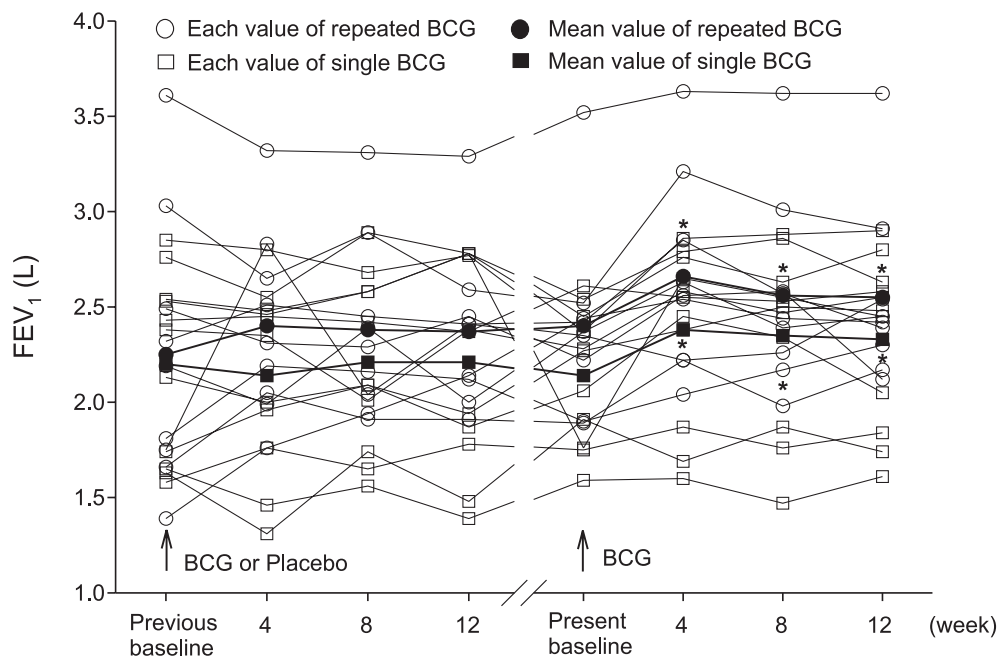


Figure 1. Forced expiratory volume in 1 s (FEV₁) measured at weeks 0, 4, 8 and 12 following Bacille Calmette–Guérin (BCG) vaccination in two groups of asthma patients who received BCG vaccine or placebo 1 year ago. **P* < 0.05 compared with the present baseline. Data were expressed as mean ± SEM. Statistical analysis was performed with the paired Student's *t*-test.

Table 2. Cytokines produced from activated peripheral blood mononuclear cell cultures after BCG vaccination

	Baseline	4 weeks	8 weeks	12 weeks
Single BCG (<i>n</i> = 11)				
IFN- γ (pg/ml)	2129.2 $\pm$ 32.5	1549.6 $\pm$ 252.2*	992.4 $\pm$ 212.6***	914.7 $\pm$ 253.3**
IL-4 (pg/ml)	16.3 $\pm$ 2.5	11.5 $\pm$ 2.8	7.9 $\pm$ 2.1**	6.9 $\pm$ 2.0***
IFN- γ /IL-4 ratio	200.3 $\pm$ 53.4	216.1 $\pm$ 43.1	164.1 $\pm$ 38.4	158.4 $\pm$ 41.3
Repeated BCG (<i>n</i> = 9)				
IFN- γ (pg/ml)	2168.6 $\pm$ 27.8	1661.1 $\pm$ 223.6*	1930.7 $\pm$ 149.3	1035.0 $\pm$ 260.7**
IL-4 (pg/ml)	21.8 $\pm$ 2.3	12.8 $\pm$ 3.3**	11.1 $\pm$ 2.3**	6.5 $\pm$ 1.8**
IFN- γ /IL-4 ratio	117.1 $\pm$ 21.9	236.8 $\pm$ 65.4*	268.2 $\pm$ 67.9*	194.0 $\pm$ 53.6

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with baseline. Data are expressed mean $\pm$ SEM. Statistical analysis was performed with the paired Student's *t*-test. BCG, Bacille Calmette-Guérin; IFN- γ , interferon- γ ; IL-4, interleukin-4.

intradermal administration of a killed *Mycobacterium vaccae* suspension was associated with improvement in atopic dermatitis. A recent clinical study (3) using *M. vaccae* failed to show any beneficial effect on asthma, but their vaccination dose was low. And BCG is more potent than *M. vaccae* in attenuating the late allergic response, airway hyperresponsiveness to methacholine, and allergic airway inflammation in BALB/c mice (4).

The increase in IFN- γ /IL-4 ratio in the repeated BCG group was lasted for only 8 weeks. As our previous report (1), the BCG-induced Th1 responses in asthma patients who were maintained under the sub-optimal medications might be overtaken after 12 weeks by the underlying Th2-type disease, with no apparent differences in the cytokine profiles. However, the apparent increase in FEV₁ in the single BCG group was accompanied by only a minimal, statistically insignificant increase in IFN- γ /IL-4 ratio at week 4. In addition, surprisingly, the actual values of both IFN- γ and IL-4 were progressively decreased following BCG vaccination. Barlan et al. (5) also showed a tendency of decrease in IFN- γ level after BCG vaccination in the supernatant of PBMC of atopic children in response to phytohemagglutinin A from 64.5 to 5.8 pg/ml, which was, however, statistically

insignificant. The BCG-infected macrophages have been shown to produce IL-10 (6), and IL-10-mediated regulatory T cell activity may have an essential role in restoring the balance of the immune system to prevent and treat allergic diseases (7). A central unmethylated C-G dinucleotide (CpG) oligodeoxynucleotides (ODNs) are known to induce Th1 responses and may inhibit established Th2 immune responses through IFN- γ and IL-10 production. The IFN- γ , induced by CpG ODNs, is also inhibited by IL-10, serving as a homeostatic mechanism for the Th1–Th2 balance (8). Taken together, IL-10 induced by BCG may downregulate IFN- γ as well as IL-4 with maintaining Th1 bias. Further investigations are needed to clarify the relationship between BCG vaccination and cytokines in asthma.

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