

Therapeutic effects of BCG vaccination in adult asthmatic patients: a randomized, controlled trial

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Background: Bacille Calmette-Guérin (BCG) vaccination in humans induces Th1 immune responses. Th1 and Th2 cells are reciprocally regulated.

Objective: To examine whether BCG vaccination of adult patients with asthma, a Th2-associated allergic disease, is clinically effective.

Methods: Forty-three moderate-to-severe asthma patients were randomly assigned into groups that received percutaneous injection of 58.2×10^7 CFUs BCG ($n = 22$) or placebo ($n = 21$) in a double-blinded fashion, on the first day of a 12-week treatment period. Medications were adjusted every 4 weeks to maintain optimal asthma control. Spirometric measurements were performed before treatment and at weeks 4, 8, and 12 after vaccination. The daily peak expiratory flow rate values, asthma symptoms, and medications were also recorded. Tuberculin skin tests, and sputum inflammatory cell and cytokine analyses were carried out before treatment and 12 weeks after vaccination.

Results: BCG vaccination significantly increased forced expiratory volume in 1 second and forced expiratory flow rate 25% to 75% at weeks 4, 8, and 12. Morning peak expiratory flow rate was significantly increased only during the first 4 weeks. Although the asthma symptom scores were not significantly changed, the weekly medication scores were significantly decreased. Tuberculin skin reactivities were significantly increased without significant alterations in induced sputum profiles. In contrast, medication scores and sputum eosinophils were significantly increased, and the interferon- γ :interleukin-4 ratio in sputum was significantly decreased in the placebo group.

Conclusions: BCG vaccination improved lung function and reduced medication use in adults with moderate-to-severe asthma. This amelioration was accompanied by a suppressed Th2-type immune response, suggesting that BCG vaccination might be an effective therapeutic modality against asthma.

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INTRODUCTION

The extensive evidence of Th2 cytokine involvement in the pathogenesis of asthma^{1,2} suggests a potential target for immunomodulation. Because the Th1 and Th2 cells are reciprocally regulated, several recent preclinical studies^{3–5} have tried to deviate and/or reverse the Th2 response into a protective Th1 response

as a potential treatment for Th2-associated allergic diseases.

Mycobacterium tuberculosis induces potent Th1 responses,⁶ and several epidemiologic studies^{7,8} have suggested that *M. tuberculosis* infection might reduce the risk of developing asthma. Bacille Calmette-Guérin (BCG), a live attenuated bovine mycobacterium, has been used in many countries since the early 20th century as a vaccine against human tuberculosis because it effectively induces cell-mediated immunity driven by Th1 cytokines. Recent experimental animal studies^{9–11} have shown that BCG infection before allergen sensitization inhibited allergen-induced airway eosinophilia and hyperresponsiveness, which are the characteristic features of asthma.

In addition, infection with BCG¹¹ or administration of heat-killed *Mycobacterium vaccae*,¹² even after allergen sensitization, inhibited an established allergic response.

In contrast, recent retrospective human studies^{13–15} failed to show a correlation between BCG vaccination during early childhood and the subsequent development of asthma or atopy. Von Hertzen and Haahtela¹⁶ suggested that only infections that induced strong cell-mediated immune responses and long-lasting memory were effective in counteracting Th2-driven diseases. Although it seems unlikely that BCG vaccination could provide long-term protection against asthma, BCG might have a suppressive effect on established allergic diseases. Because the financial costs of asthma management are substantial and many asthmatic patients respond poorly to conventional therapies, the development of new, inexpensive, and more effective therapeutics is needed.

Because BCG suppressed established allergic responses¹¹ as well as the development of allergic responses in animal models, we examined the clinical efficacy of BCG vaccination in adult asthmatic patients.

METHODS

Patients

Adult asthma patients were recruited from the Allergy Outpatient Clinic, Chonnam University Hospital, Kwangju, Korea. All patients had suffered from asthma for more than 1 year, had used asthma medications daily, fulfilled the criteria for asthma set down by the American Thoracic Society,¹⁷ and had moderate-to-severe perennial asthma.¹⁸ All patients with severe persistent asthma included in the study were reluctant to take oral prednisolone, fearing the side effects associated with sys-

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temic steroids. The subjects were clinically stable for at least 4 weeks before the start of the study. Candidates were excluded from the study based on these criteria: chronic use of oral corticosteroids, malignancies, cell-mediated immune deficiency, positive human immunodeficiency virus serology, severe dermatologic disease, severe nutritional deficiency, rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, multiple sclerosis, previous specific immunotherapy, pregnancy, or lactation. The study was approved by the ethical review board of Chonnam University Hospital. All patients were informed about the experimental procedures and provided written informed consent.

Study Design

The study was performed as a prospective, double-blinded, randomized, placebo-controlled trial between April and October 2000. During a 4-week run-in period, we carried out objective (spirometry and peak expiratory flow rate [PEFR] monitoring) and clinical (asthma symptoms and medication scores) measurements of asthma severity, evaluations of previous mycobacterial infection, including tuberculin skin tests, allergy skin tests for common aeroallergens, and inflammatory cell and cytokine analyses on induced sputum. After the run-in period, the patients were randomly divided into two groups and given either BCG or placebo (saline). Where necessary, patients inhaled a short-acting β_2 -agonist (salbutamol) as a rescue medication, with or without a short course of oral prednisolone (20 to 30 mg daily). To maintain optimal conditions, the patients' regular (long-term control) asthma medications were adjusted in a blinded fashion every 4 weeks, according to the guidelines for the diagnosis and management of asthma set forth in Expert Panel Report 2.¹⁸ The inevitable development of erythema at the vaccination site in the BCG-administered group posed a problem for the maintenance of a double-blinded trial. To overcome this, the study coordinator (I.S.C.) adjusted the asthma medica-

tions without examining the vaccination site and the other investigator (Y.I.K.) monitored adverse reactions. Further, all patients were given to believe that BCG vaccination using an expensive Japanese needle cylinder generally produced minimal or no reaction; none of the patients questioned whether they had received BCG vaccination. The objective and clinical measures were repeated at each visit, and at the end of the 12-week treatment period, tuberculin skin tests and inflammatory cell counts and cytokines in induced sputum were analyzed.

Past Exposure to Mycobacteria

Previous exposure to mycobacteria was judged by the appearance of healed tubercular lesions in chest radiographs, a history of previous antituberculosis chemotherapy, the presence of an old BCG scar in the deltoid area, or on the basis of a positive tuberculin skin test. All subjects with BCG scars had received BCG vaccination at 8 to 10 years of age. The chest radiographs were interpreted independently by two radiologists. The tuberculin skin tests were performed using the Mono-Vacc Test (old tuberculin) unit (Pasteur Merieux, Lyon, France) by a single operator who was blinded to the treatment regimens. The skin was held taut as the loaded points were pressed firmly into a cleaned site on the volar surface of the midforearm so that the punctures and an imprint of the base were visible. Readings were done 48 to 72 hours later. The test was shown to be reproducible in a preliminary study with 25 healthy adults. For intraindividual reproducibility, the κ coefficient was 0.89 ($P < 0.001$) and the Pearson correlation was 0.98 ($P < 0.001$), and for interindividual reproducibility, the κ coefficient was 0.78 ($P < 0.001$), and the Pearson correlation was 0.92 ($P < 0.001$).

Allergy Skin Tests

Allergy skin-prick tests were carried out with 15 common aeroallergens. Histamine dihydrochloride (1 mg/mL) and saline were used as positive and negative controls, respectively. The al-

lergen test kit included pollen from trees (alder, birch, hazel), grasses (bermuda, timothy), and weeds (mugwort, ragweed); molds (*Alternaria*, *Aspergillus*, *Penicillium* spp.); dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*); animal dander (cats, dogs); and cockroach components (Bencard, Brentford, England). The longest perpendicular diameter of each wheal was measured with a millimeter ruler after 15 minutes, and the arithmetic means were calculated. Subjects were classified as atopic if the application of the allergen produced a wheal 3 mm or larger in diameter than that seen in a negative control.

BCG Vaccination

Vaccination involved the spreading of 0.15 mL (containing 58.2×10^7 CFUs) of BCG vaccine (Tokyo-172 strain; Japan BCG laboratory, Tokyo, Japan) or placebo, on a 3 cm $\times$ 1.5 cm patch of intact skin in the deltoid area. The epidermis in the patch area was then perforated twice to a depth of approximately 2 to 3 mm using the Japanese Needle-Planted Cylinder (Japan BCG Laboratory, Tokyo, Japan).

Clinical Assessments

Asthma symptoms and all medications, including supplemental β_2 -agonist and prednisolone, were recorded for each patient. Every morning (6:00 to 8:00 AM) and evening (4:00 to 6:00 PM) PEFRs were monitored with a Ferraris PEFR meter (Ferraris Medical, CA), and the best of three separate measurements was selected as representative of that time-point. The forced expiratory volume in 1 second (FEV₁) and the forced expiratory flow 25% to 75% (FEF_{25%-75%}) were measured with a spirometer (Spiro Analyzer ST-250, Fukuda Sangyo, Tokyo, Japan) at the same time of day on every patient visit. Patients were instructed to refrain from inhaling short-acting β_2 -agonists (eg, salbutamol) for at least 6 hours before the test. The largest FEV₁ value was selected from three or more acceptable maximal expiratory flow volume curves and FEF_{25%-75%} was deduced from the best test curve, according to

recommendations published by the American Thoracic Society.¹⁹ We monitored local (vaccination-site) and systemic adverse reactions by physical examination at each visit or via phone contact with individual patients.

Sputum Induction and Analysis

Patients inhaled 200 μ g of salbutamol and 10 minutes later inhaled 4.5% saline for 15 minutes via an ultrasonic nebulizer (Ultra-neb 2000, DeVilbiss, Somerset, PA) to induce sputum. Sputum was processed as previously described.²⁰ Briefly, opaque sputum portions were selected and digested with 0.1% dithiothreitol (Sigma, St. Louis, MO), and filtered through 53- μ m nylon gauze (Spectrum Laboratories Inc, Savannah, GA). Total cell counts were determined and cytospin (CF-120, Tokyo, Japan) slides were prepared. Differential cell counts were expressed as the percentage of nonsquamous cells in a sample of 300 cells on each of two slides stained with Diff-Quik (American Scientific Products, McGaw, IL). The sample was considered appropriate when squamous cell contamination was <20%.²¹ The interferon (IFN)- γ and interleukin (IL)-4 levels in sputum were measured using commercially available enzyme-linked immunoadsorbent assay kits (IFN- γ : Endogen, Woburn, MA; IL-4: Quantikine HS, R&D Systems, Minneapolis, MN).

Statistical Analyses

The primary endpoints were changes in FEV₁, FEF_{25%-75%}, and morning PEFR above the baseline values measured at the end of the run-in period. Secondary endpoints included changes in symptom scores, medication scores, tuberculin skin reactivities, sputum eosinophil counts, or in sputum IFN- γ and IL-4 concentrations. The baseline values for each of the variables recorded in the diaries were determined as the averages or sums of weekly values for week 4 of the run-in period. Categorical variables were compared using Fisher's exact test for intergroup differences and McNemar's test for intragroup differences. The differences in continuous variables between the

two groups were analyzed with the unpaired Student's *t* test for parametric data and the Mann-Whitney *U* test for nonparametric data. The differences within the same group were analyzed using the paired Student's *t* test for parametric data and the Wilcoxon's signed-rank test for nonparametric data. A *P* < 0.05 value was considered statistically significant.

RESULTS

Of the 49 patients who participated in the run-in period, 43 completed the study; 3 patients withdrew because of family concerns, 1 because of a dental problem, and 2 because of missed clinical visits.

The patient demographics and baseline characteristics are shown in Table 1. The characteristics were similar for the two groups, although the prevalences of BCG vaccination scarring in the BCG-treated group and of previous antituberculosis chemotherapy in the placebo group were significantly higher than in the other group. The number of positive tuberculin tests was not significantly different between the two groups and all subjects showed evidence of past exposure to mycobacteria. There were no significant differences between the groups in the numbers of patients with atopy and sensitization to seasonal pollens or with rhinitis. All subjects with rhinitis had used nasal steroids for at least 4 weeks before beginning the study and were clinically stable during the 12-week treatment period.

None of the placebo group and 2 of 22 (9%) of the BCG group reported soreness at the site of vaccination. Erythema, manifested as macule or maculopapule, occurred in all of the BCG-administered subjects, but was not observed in any of the patients who received the placebo. Neither group showed evidence of ulceration, drainage, or lymphadenitis. The skin reactions in the BCG group faded more than 12 weeks and 3 of 22 (13.6%) of the patients in this group showed near-normal skin by week 12.

Pulmonary Function

BCG vaccination significantly increased FEV₁ values from the baseline level (mean $\pm$ SD) of 2.16 ± 0.57 L to 2.43 ± 0.49 , 2.42 ± 0.51 , and 2.39 ± 0.52 L at weeks 4 (*P* < 0.01), 8 (*P* < 0.01), and 12 (*P* < 0.05), respectively. In contrast, the FEV₁ levels were not significantly changed in the placebo group at these time-points. There were significant differences in FEV₁ changes from baseline between the groups at each time-point (Fig 1A). Similarly, BCG vaccination significantly increased FEF_{25%-75%} from the baseline level of 1.43 ± 0.72 L/second to 1.83 ± 0.72 , 1.82 ± 0.74 , and 1.80 ± 0.72 L/second at weeks 4 (*P* < 0.01), 8 (*P* < 0.001), and 12 (*P* < 0.01), respectively. In addition, the changes in FEF_{25%-75%} at each time-point were significantly different between the groups (Fig 1B).

BCG vaccination also significantly increased morning PEFR (weekly average) from 427 ± 93 L/minute to 439 ± 92 , 441 ± 92 , 445 ± 98 , and 447 ± 102 L/minute at weeks 1 (*P* < 0.05), 2 (*P* < 0.05), 3 (*P* < 0.01), and 4 (*P* < 0.05), respectively. The differences in morning PEFR changes between the groups were significant at weeks 3 and 4 (Fig 2A). Evening PEFR was not significantly changed from the baseline value in either group.

Asthma Symptoms and Medication Scores

The mean weekly asthma symptom scores²² were decreased significantly (*P* < 0.05) from 5.43 ± 13.3 to 2.25 ± 7.4 , only at week 7 of BCG vaccination, and the changes in symptom scores were not significantly different between the groups (Fig 2B). However, the mean weekly total medication scores²³ were significantly decreased from 123 ± 40 to 114 ± 38 , 113 ± 38 , 111 ± 39 , and 110 ± 40 at weeks 4 (*P* < 0.05), 5 (*P* < 0.05), 6 (*P* < 0.01), and 7 (*P* < 0.01) of BCG vaccination, respectively. In the placebo group, the mean weekly total medication scores were increased from 105 ± 45 to 111 ± 46 , 113 ± 45 , and 121 ± 59 at weeks 5, 6, and 7 (*P* < 0.05), respec-

Table 1. Baseline Characteristics of the 43 Asthmatic Subjects Randomly Assigned to Receive BCG or Placebo

Characteristic	BCG (n = 22)	Placebo (n = 21)
Age (years)	44.9 ± 10.8	50.7 ± 9.4
Sex (M/F)	10/12	12/9
Duration of asthma (years)	9.4 ± 6.7	8.0 ± 9.0
Rhinitis (%)	7 (31.8)	6 (28.6)
Evidence of past exposure to mycobacteria (%)		
Old healed tuberculosis lesion on chest radiographs	7 (31.8)	13 (61.9)
History of antituberculosis chemotherapy	0 (0)**	6 (28.6)
Previous BCG vaccination scar	17 (77.8)*	9 (42.9)
Positive tuberculin test (≥5 mm skin induration)	13 (59.1)	18 (85.7)
Any one of previous four characteristics	22 (100)	21 (100)
Skin prick test (%)		
Atopy	14 (63.6)	15 (71.4)
Sensitization to tree pollen	4 (18.2)	6 (28.6)
Sensitization to grass pollen	2 (9.1)	1 (4.8)
Sensitization to weed pollen	4 (18.2)	4 (19.0)
FEV ₁ (L)	2.2 ± 0.6	2.4 ± 0.6
(% of predicted value)	69.3 ± 15.7	74.5 ± 10.5
FEF _{25-75%} (L/sec)	1.4 ± 0.7	1.6 ± 0.7
(% of predicted value)	42.1 ± 19.1	49.3 ± 17.7
PEFR (L/min)		
Morning	426.9 ± 92.5	440.8 ± 141.4
Evening	435.7 ± 90.5	448.6 ± 139.1
Weekly symptom score	5.4 ± 13.3	6.2 ± 12.1
Weekly medication-use score	123.2 ± 39.9	104.6 ± 44.8
Regular medications (%)		
Theophylline	22 (100)	18 (85.7)
Oral β ₂ -agonist	3 (13.6)	5 (23.8)
Leukotriene receptor antagonist	15 (68.2)	11 (52.4)
Inhaled long-acting β ₂ -agonist	19 (86.4)	19 (90.5)
Inhaled corticosteroid	22 (100)	20 (95.2)

Plus-minus values are means ± SD.

* $P < 0.05$.

** $P < 0.01$ vs placebo. Statistical analyses were performed using the unpaired Student's t test for continuous variables and Fisher's exact test for categorical variables.

tively. The degree of change in medication scores was significantly different between the groups during weeks 1 to 10 (Fig 2C). When medication use was subdivided into the categories of controller (sustained-release theophylline, pranlukast hydrate, bambuterol HCl, salmeterol xinafoate, and fluticasone propionate) or rescue (salbutamol and prednisolone) treatments, the mean weekly controller medication scores were significantly below the baseline level in the BCG group at weeks 5 ($P < 0.05$), 6 ($P < 0.05$), 7 ($P < 0.01$), 8 ($P < 0.01$), 9 ($P < 0.05$), 10 ($P < 0.05$), 11 ($P < 0.05$), and 12 ($P < 0.01$). In contrast, the mean weekly controller medication scores

were above the baseline in the placebo group at weeks 5 ($P < 0.05$), 6 ($P < 0.05$), and 7 ($P < 0.01$). Changes in the controller medication scores from baseline were significantly different between the BCG and placebo groups during weeks 1 to 12 ($P < 0.05$). However, the mean weekly rescue medication scores were not significantly different from baseline in either group. Change in each medication-use score from baseline was significantly different between the groups at weeks 6 and 7 in theophylline and in inhaled long-acting bronchodilator ($P < 0.05$), and during weeks 5 to 12 in inhaled corticosteroid ($P < 0.05$). Among the BCG-vaccinated patients, the inhaled

steroid dosage was decreased in 1 of 22 (4.5%) at week 1, 2 of 22 (9.1%) at week 3, 3 of 22 (13.6%) at week 4, 4 of 22 (18.2%) at week 6, and 5 of 22 (22.7%) at week 9. In contrast, among the placebo-vaccinated patients, the steroid dosage was decreased in only 1 of 21 (4.8%) patients by week 9.

Delayed Hypersensitivity to Tuberculin

The skin induration size after vaccination was significantly increased at 12 weeks compared with before vaccination (10.5 ± 8.9 mm vs 7.55 ± 7.8 mm; $P < 0.05$) in the BCG group, whereas it was significantly decreased in the placebo group (10.6 ± 11.4 mm vs 15.1 ± 15.4 mm; $P < 0.05$). The proportion of patients with positive tuberculin response (≥ 5 -mm skin induration) after vaccination was significantly higher than before vaccination (22 of 22, 100% vs 13 of 22, 59.1%; $P < 0.01$) in the BCG group, whereas, in this respect, there were no significant differences in the placebo group (14 of 21, 66.7% vs 18 of 21, 85.7%; $P = 0.125$).

Inflammatory Cells and Cytokines in Induced Sputum

The adequate sputum samples both before and after vaccination were obtained from 15 patients in the BCG group and 13 patients in the placebo group. There were no significant differences in the baseline values between the two groups. The differential cell counts and cytokine profiles in the sputum of BCG group patients were not altered from the baseline after 12 weeks. However, the placebo group showed a small but significant increase in eosinophil counts ($P < 0.05$), a significant decrease in IFN- γ ($P < 0.05$), a significant increase in IL-4 ($P < 0.05$), and a significant decrease in the IFN- γ :IL-4 ratio ($P < 0.01$; Table 2).

DISCUSSION

In this study, BCG vaccination significantly increased pulmonary function and reduced asthma medication use in adult patients with moderate-to-severe asthma. A beneficial effect of BCG vaccination on asthma has been noted

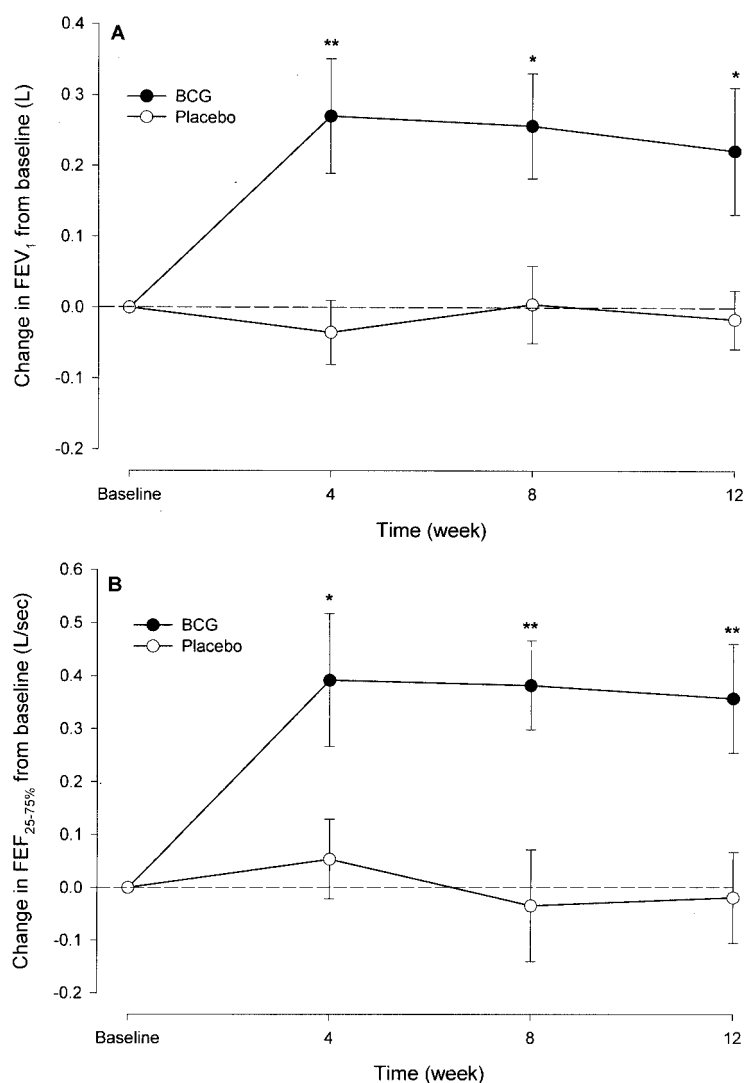


Figure 1. Mean ($\pm$ SE) changes from the baseline levels in FEV₁ (A) and FEF_{25-75%} (B) at weeks 4, 8, and 12 after vaccination. * $P < 0.05$ and ** $P < 0.01$ compared with the placebo group. Statistical analysis was performed with the unpaired Student's *t* test.

in epidemiologic^{7,8} and animal laboratory⁹⁻¹² studies. Nevertheless, many researchers^{16,24} remain skeptical about the clinical efficacy of such a treatment. This is the first study showing a therapeutic effect of BCG on human asthma.

In retrospective human studies,¹³⁻¹⁵ which failed to demonstrate protection against the development of asthma or atopy, BCG vaccinations were performed during early childhood and the effects were evaluated several years later. Therefore, as suggested by von Hertzen and Haahtela,¹⁶ it is likely that

the relatively weak cell-mediated immune responses induced by BCG vaccination were insufficient to sustain long-term protection against asthma. Although BCG vaccination has been reported to offer more than 70% protection against tuberculosis for at least 10 years,²⁵ repeated exposure to different allergens over a prolonged period might abrogate the protective effects of BCG vaccination.

The apparent beneficial effects shown in the present study may not be reproducible in Western societies

where tuberculosis prevalence is very low and BCG vaccination is not routinely performed. As tuberculosis prevalence in Korea is high and most Koreans receive BCG vaccination in childhood, all patients in this study showed evidence of past exposure to mycobacteria. Therefore, in these cases, BCG vaccination might have had a booster effect. Given that the effects of BCG vaccination are maximal at 8 to 16 weeks postvaccination,²⁶⁻²⁹ we were surprised to observe a significant improvement in morning PEFR 1 week after BCG vaccination. This phenomenon may be attributable to the booster effect, described by Ravn et al.²⁶ in which previously sensitized patients mount a potent and highly accelerated recall response within 1 week of BCG vaccination.

Although the improvements in FEV₁ and FEF_{25-75%} were maintained for 12 weeks after BCG vaccination, the improvements in morning PEFR were not sustained for more than 4 weeks after vaccination. One possible explanation for this discrepancy is that most moderate-to-severe asthmatic patients are morning-dippers and thus, an early deterioration in asthma disease may begin with a decrease in the morning PEFR, in the absence of a concomitant decrease in the daytime spirometric measurement. However, the effect of BCG on morning PEFR might be masked in this study because asthma medication use had been gradually reduced. Despite the reductions in medication, the absence of decrease below baseline value in morning PEFR may also indicate an effect of BCG. These results may also explain the apparent lack of improvement in asthma symptoms. Additionally, all patients who participated in this study had maintained control over their asthma with long-term control medications.

Vaccination-site erythema occurred only in the BCG group and may have influenced the blinding of the study. In a worst-case scenario, if the coordinator adjusted medications intentionally, the medication scores would have had to be increased to improve lung function in the BCG group and decreased

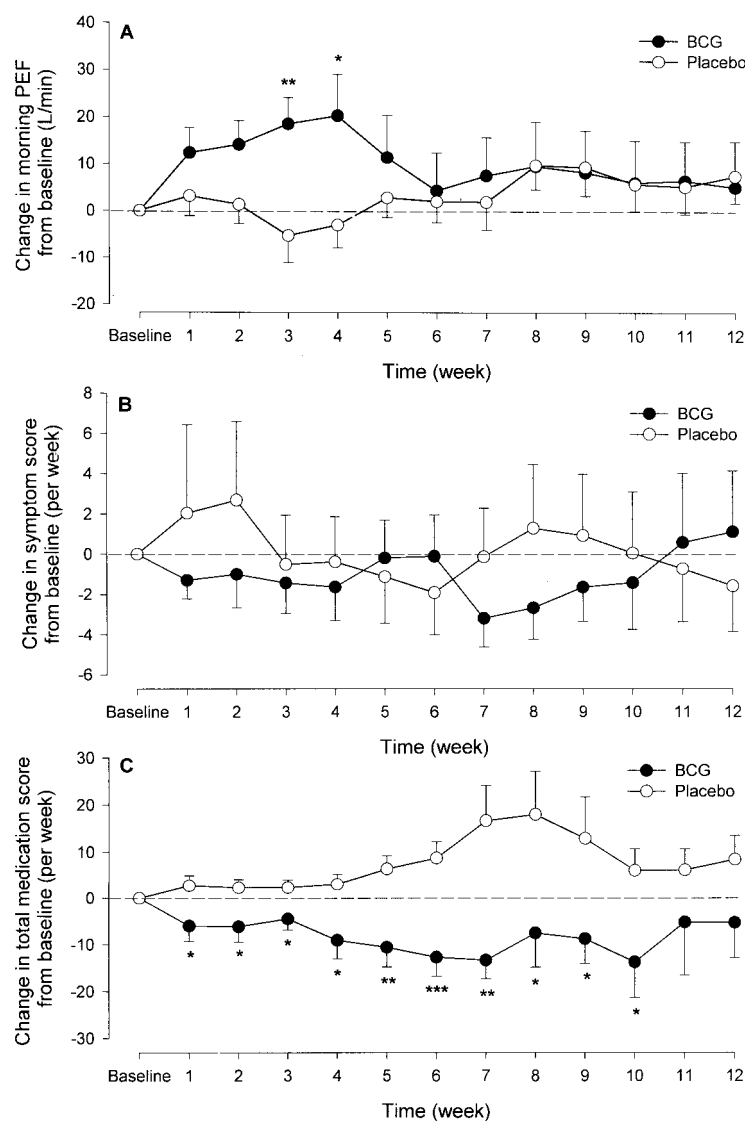


Figure 2. Mean ($\pm$ SE) changes from the baseline levels in morning PEF (A), weekly asthma symptom score (B), and weekly total asthma medication score (C). The representative PEF values were determined as weekly averages. The asthma symptoms²² included: dyspnea on a scale of 0 to 9 (0, no symptoms; 1, trivial; 3, mild; 6, moderate; 9, severe); cough on a scale of 0.5 to 1 (0.5, infrequent; 1, frequent); and night sleep disturbance on a scale of 0 to 12 (0, slept well; 4, slept reasonably well despite dyspnea; 8, slept with difficulty; 12, kept awake). For scoring asthma medications,²³ each of the following was designated as one unit: one puff from salbutamol inhaler, a 100-mg sustained-release theophylline capsule, a 112.5-mg pranlukast hydrate capsule, a 5-mg bambuterol HCl tablet, a half-puff from a salmeterol xinafoate inhaler, a half-puff of fluticasone propionate, or a 0.5-mg prednisolone tablet. The severity of symptoms and medication requirements were determined as total weekly scores. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with the placebo group. Statistical analysis was performed with the unpaired Student *t* test.

to deteriorate in the placebo group; the reverse was in fact the case. These would reduce, albeit incompletely, any unblinded effect. An alternative control

group, receiving a different immunogen, might be used in future studies.

It has been shown that BCG vaccination induces Th1 responses in hu-

mans.^{26–29} The IL-12 and IFN- γ cytokines, produced by the interaction between mycobacterial lipoproteins and macrophage Toll-like receptors,³⁰ repressed Th2-driven mechanisms in animal experiments.^{3,4,31} The production of IL-10 by BCG-infected monocyte-derived macrophages³² may downregulate Th2 clones and allergic inflammation.³³ Because the tuberculin reaction has been reported to correlate well with IFN- γ responses after BCG vaccination,²⁹ our finding that the tuberculin reaction was significantly increased after BCG vaccination corroborates previous evidence that BCG vaccination induces Th1 responses and suggests that the clinical effects of BCG on asthma are related to a shift in the Th1/Th2 balance. However, the cytokine profiles at 12 weeks after BCG vaccination were not different from the baseline values. Possible explanations for this discrepancy include the suboptimal asthma treatment received by patients because of concerns about the potential side effects of systemic steroids, and the measurement of followup cytokine levels at inappropriate times. We found increased medication requirements, sputum eosinophils, and deviations in the Th1/Th2 balance to predominant Th2-type in the placebo group, but not in the BCG group. These results suggest a suppressive effect of BCG on ongoing asthma in patients who were maintained on suboptimal, long-term control asthma medications. In this situation, the BCG-induced Th1 responses might be overtaken after 12 weeks by the underlying Th2-type disease, with no apparent differences in the cytokine profiles. Further investigations into the systemic Th1/Th2 responses, including the production of cytokines by peripheral blood T cells, are needed.

Based on the differentiation of Th0 cells into Th1 or Th2 cells, many researchers^{13–15} have concentrated on the prevention of asthma or atopy with BCG vaccination during early childhood. Although chronic antigen stimulation has been found to render the T cell populations more homogenic and resistant to change,³⁴ reversals

Table 2. Sputum Cell Counts and Cytokine Levels Before and After BCG or Placebo Vaccination

	BCG (n = 15)		Placebo (n = 13)	
	Baseline	12 wks	Baseline	12 wks
Eosinophils (%)	4.9 ± 14.4	3.0 ± 2.1	1.7 ± 1.7	3.0 ± 1.7*
Neutrophil (%)	42.9 ± 24.8	39.6 ± 17.9	34.0 ± 20.8	37.0 ± 19.1
Lymphocyte (%)	2.1 ± 2.3	2.0 ± 1.4	2.3 ± 3.0	3.9 ± 3.8
Macrophage (%)	42.3 ± 25.0	46.5 ± 18.3	57.7 ± 19.0	48.0 ± 14.7
Columnar cell (%)	7.9 ± 6.7	8.9 ± 10.5	4.2 ± 4.8	8.1 ± 10.5
IFN- γ (pg/mL)	38.2 ± 18.8	27.3 ± 16.7	35.6 ± 24.7	20.3 ± 8.9*
IL-4 (pg/mL)	0.8 ± 0.5	0.8 ± 0.4	0.8 ± 0.2	1.3 ± 0.9*
IFN- γ :IL-4 ratio	65.6 ± 48.3	38.1 ± 27.4	45.5 ± 26.5	21.5 ± 18.8**

Values were expressed as mean $\pm$ SD.

* $P < 0.05$.

** $P < 0.01$ versus baseline levels in the placebo group. Statistical analyses were performed using the Mann-Whitney U test for intergroup and Wilcoxon's signed-rank test for intragroup differences.

have been shown to occur in animals even after the establishment of Th2 clones.^{3-5,35} Further, patients who underwent rush immunotherapy with house-dust mite extract showed an increase in peripheral blood lymphocytes producing IFN- γ .³⁶ A recent investigation by Arkwright and David³⁷ describing the efficacy of *M. vaccae* in atopic dermatitis subjects corroborates the results of our study in several regards. Thus, BCG vaccination of patients with an established allergic disease, such as asthma, probably activates the remaining Th1 cells to overcome the Th2-driven disease.

In the present study, the baseline FEV₁ and FEF_{25%-75%} values were slightly lower in the BCG group than in the placebo group, which suggests that patients in the BCG group had less controlled asthma and might have been more responsive to asthma medications. However, the differences between the two groups were not statistically significant. In addition, all patients were clinically stable for at least 4 weeks before vaccination. Further, the medication scores were significantly decreased after BCG vaccination, but slightly increased after placebo administration, indicating that lung function in the BCG group may have been increased by BCG vaccination rather than by medication. Asthma is an inflammatory disease affecting both the large and small airways. Gen-

erally, it is known that PEFR reflects the function of the large airways, FEF_{25%-75%} the small airways, and FEV₁ both the large and small airways. In our study, the significant increases in both FEV₁ and FEF_{25%-75%} after BCG vaccination suggest that BCG vaccination may be effective in improving the functions of both large and small airways in cases of asthma.

CONCLUSION

BCG vaccination is relatively safe. Further, the BCG vaccination administered via the percutaneous route in this study is generally associated with lower complication rates compared with vaccination via the intradermal route.²⁹ Although increased Th1 responses have been correlated with autoimmune disease,³⁸ there is still no study reporting the relationship between conventional BCG vaccination and autoimmunity. Interestingly, BCG vaccination has led to clinical remission in patients with type I diabetes.³⁹ The safety, cost, and efficacy of the BCG vaccine may be sufficient to justify therapeutic trials in patients with asthma.

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