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Personal Factors Affecting Therapeutic Responses to BCG Vaccination in Asthmatics

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Purpose: Bacille Calmette-Guérin (BCG) vaccination has been reported to be an effective treatment for asthma in several animal models. This study investigated whether the response to BCG treatment in asthma depends on subject clinical characteristics. **Methods:** Stable asthma patients were vaccinated with BCG. One month later, alterations in pulmonary function after vaccination and their relationships with subject clinical characteristics were determined. **Results:** Of 149 patients with asthma, 54 (36.2%) showed a good or fair response to BCG. The ΔFEV1 after vaccination was significantly related to age (r=-0.348, $P<0.001$), peripheral blood eosinophil counts (r=0.315, $P<0.001$) and baseline FEV1, expressed as % personal best value (r=-0.474, $P<0.001$), but not to FEV1 % predicted value (r=-0.066, $P>0.05$). A good/fair response was highly prevalent in atopic females compared with atopic males, especially among those aged ≤50 years (90.9% vs. 40.0%, $P=0.024$). Age ($P<0.001$, odds ratios (OR)=0.92, confidence interval (CI)=0.88-0.96) and atopy ($P<0.01$, OR=4.95, CI=1.70-14.44) were significant predictors for a good/fair response in females. However, blood eosinophil counts ($P<0.05$, OR=1.18, CI=1.01-1.39) and FEV1 % best ($P<0.001$, OR=0.86, CI=0.79-0.94), but not age or atopy, were significant predictors in males. Approximately three-quarters of the males were smokers. **Conclusions:** The therapeutic effect of BCG in asthma may differ according to patient clinical characteristics. The greatest benefit occurred in young atopic females. Asthma activity indices, such as eosinophilia and FEV1 % best, were more predictive of a good/fair response in males; this may have been related to cigarette smoking.

Key Words: Activity; asthma; atopy; BCG; gender; smoking

INTRODUCTION

The main reason for the recent increase in the prevalence of asthma may be inadequate differentiation of naïve T cells to Th1 and regulatory T (Treg) cells, itself induced by a decrease in infectious disease due to improved hygiene.[1] The human immune system tends to deviate to Th2 immune reactions, which are suppressed by Th1 and Treg cells. When this suppression is inadequate, Th2 immune reactions are activated and allergic diseases can result (the so-called "hygiene hypothesis"). In this context, numerous animal studies have reported that bacille Calmette-Guérin (BCG) vaccination can prevent or treat asthma by inhibiting airway hyperresponsiveness (AHR) and eosinophilic airway inflammation through the induction of Th1/Treg reactions.[2-6] Our previous study indicated that BCG vaccination improved pulmonary function and reduced the requirement for therapeutic agents in patients with asthma.[7] In contrast, Shirtcliffe et al.[8] reported that repeated heat-inactivated BCG vaccinations had no additive therapeutic effect and induced severe local reactions. This may be because the effect of heat-killed BCG in the development of airway eosinophilia is lower than that of live BCG,[9] and/or that the BCG Tokyo 172 strain has a greater therapeutic effect than other strains.[10]

Because responses to drug therapy differ among individual patients, and BCG exerts its therapeutic effect by inhibiting Th2 immune reactions, it is possible that BCG vaccination has a higher therapeutic effect in atopic patients with asthma. Indeed, in peripheral blood mononuclear cells (PBMCs) taken from atopic, but not non-atopic, patients with asthma, BCG sig-

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nificantly inhibits production of interleukin (IL)-5.[11] Allergic asthma is a disease characterized by eosinophilic airway inflammation and AHR caused by inappropriate Th2 immune reactions, resulting from exposure of an atopic individual to a sensitized allergen. For this reason, the BCG vaccine may be effective during active immune and inflammatory reactions, but may be ineffective during their remission due to the presence of only remnant remodeling with irreversible airflow obstruction. Additionally, Cui et al.[12] reported that male asthmatic mice produced more interferon (IFN)-γ and less IL-4/ovalbumin-specific IgE than female asthmatic mice and that BCG treatment showed a lesser therapeutic effect in male mice. Moreover, Lazarus et al.[13] suggested that the therapeutic effect of inhaled steroids was lower in smokers than in non-smokers. However, to our knowledge, there is no reported study of the effect of these variables on BCG vaccination in asthmatic patients. Thus, here we evaluated the therapeutic effect of BCG vaccination in terms of asthmatic patients' clinical characteristics.

MATERIALS AND METHODS

In total, 153 adult patients with asthma visited the allergy clinic of two university hospitals and were administered the BCG vaccine. A diagnosis of asthma was established in patients who met the following criteria: (1) presence of asthma symptoms, (2) improvement in forced expiratory volume in 1 second (FEV1) by ≥12% and ≥200 mL after asthma treatment[14] or <8 mg/mL of methacholine required to decrease FEV1 by 20% (PC20) in a methacholine bronchoprovocation test.[15] All subjects received asthma treatment for ≥6 months and were stable for ≥1 month and had 40-79% of their personal best or predicted FEV1 value, and a FEV1/forced vital capacity (FVC) ratio of <80%. This study included 22 subjects who received the BCG vaccine in the 2002 clinical trial[7] and 11 in the 2003 clinical trial.[16]

This study was approved by the Institutional Review Boards of both university hospitals (IRB No. E-2008-05-036) as well as the Korean Food and Drug Administration. Written informed consent was obtained from each patient.

The therapeutic effect of the BCG vaccine was assessed without changing asthma treatment modalities. BCG vaccination was performed using 5.8×10^8 CFU of the Tokyo-172 strain (Japan BCG Laboratory, Tokyo, Japan). The vaccine was administered twice at a 2-3 mm depth in the skin of the upper arm using a Japanese Needle-Planted Cylinder (Japan BCG Laboratory). A good responder was defined as a subject who had an increase in FEV1 of ≥12% and ≥200 mL[14] or a peak expiratory flow (PEF) of ≥20% and 60 L/min,[17] 1 month after BCG vaccination. A fair responder was defined as a subject who had an FEV1 of ≥6% and ≥100 mL or a PEF of ≥10% and 30 L/min 1 month after BCG vaccination, and a poor responder was defined as a subject with any other test result 1 month after BCG vaccination. The degree of decrease in the present FEV1 from the personal best value within 1 year of BCG vaccination was rated on an arbitrary point scale with intervals of 6%. In cases where FEV1 % personal best could not be obtained, the minimum PEF expressed as a percentage of the best (Min%Max)[14] during the 1-4 weeks prior to BCG vaccination was rated on a point scale with intervals of 10%. Asthma activity was defined as a decrease in FEV1 of ≥6% or in PEF of ≥10% and the activity grade was expressed as a point.

Pulmonary function tests were performed using a computerized spirometer (Spiro Analyzer ST-250, Fukuda Sanyo, Tokyo, Japan). Representative values for spirometry were selected from the best spirogram having the highest FEV1 plus FVC values from three or more spirograms according to the Manual of the Intermountain Thoracic Society.[18] The regression equation of Crapo et al.[19] was used for calculating predicted values of FEV1 and FEV1/FVC ratio. Spirometry was performed at least 4 hours after discontinuing rapid-acting β_2-agonist inhalers. Post-vaccination tests were conducted within 1 hour of the time points in a day of the tests conducted prior to BCG vaccination.

White blood cell counts and the percentage and numbers of eosinophils (white blood cell count×eosinophil %) in peripheral blood were measured. Elevated eosinophil levels (eosinophilia) were defined as ≥450/mm^3.[20] Additionally, serum IgE levels (normal <100 IU/mL) were measured using a nephelometer (Behring Diagnostics GmbH, Frankfurt, Germany). Skin prick tests were performed using 55 common allergens, including *Dermatophagoides farinae* and *D. pteronyssinus* (Bencard, Brantford, UK). Histamine solution (1 mg/mL) and 0.9% saline were used as positive and negative controls, respectively. A positive reaction was recorded when the value of the averaged wheal diameter of an allergen divided by that of histamine was ≥25%. IgE values specific for *D. farina*, *D. pteronyssinus*, *Aspergillus fumigates*, *Penicillium* and cockroach were measured using a UniCAP 100^e (Pharmacia Diagnostics, Uppsala, Sweden). A positive value was defined as ≥0.35 kU/L. Atopy was defined as a positive skin prick test or serum IgE level for any allergen. Sensitization to house dust mites was defined as a positive skin prick test for either of the two house dust mites.

Three subjects who were lost to follow-up 1 month after BCG vaccination, and one subject who was treated for pneumonia, were excluded from the study. Data are expressed as mean±SE. In each group, comparisons between pre- and post-vaccination values were made using a paired *t*-test. Comparisons between individual groups were made with Student's *t*-test, ANOVA, *post hoc* analysis using the Tukey test, the chi-squared test, and the Fisher's exact test, as appropriate. Correlations between individual valuables were determined using Spearman rank correlation coefficients. The odds ratios of good/fair responses were obtained using a logistic regression model. A *P* value of <0.05 was considered to indicate statistical significance.

RESULTS

In total, 149 subjects (80 males, 69 females) were included. No significant difference was noted between males and females in age, duration of asthma, or associations with allergic rhinitis or paranasal sinusitis (Table 1). Peripheral blood eosinophil % was significantly higher in females, while the frequency of smoking, atopy or sensitization to house dust mites was significantly higher in males. Before BCG vaccination, FEV1 % predicted was significantly higher; however, FEV1 % personal best values were lower in females. Also, after BCG vaccination, FEV1/FVC ratio % predicted was significantly higher, but FEV1 % personal best values were lower in females. After BCG vaccination, activity grade was significantly higher in females. After BCG vaccination, Min%Max in males and FEV1/FVC ratio and Min%Max in females were significantly increased. A higher trend towards good/fair responses was observed in female than in male subjects (39.1 vs. 33.8%), but this difference was not statistically significant.

Patients with active asthma were significantly younger, and had a significantly lower prevalence of smoking (Table 2). Both before and after BCG vaccination, FEV1 % personal best and Min%Max were significantly lower, but the degree of improvement in FEV1 after BCG vaccination was significantly higher in subjects with active asthma. The good/fair response rate was also significantly higher in subjects with active asthma (22.2%/20.2% vs. 12.5%/7.5%; $P<0.05$). In subjects with active asthma, FEV1 % personal best and Min%Max were significantly in-

Table 1. Clinical characteristics of subjects with asthma and their response to BCG vaccination

	Male (n=80)	Female (n=69)
Age (yr)	58.6±1.4	56.0±1.7
Smoker*	58 (73.4%)	4 (5.8%)
Duration of asthma (yr)	12.3±1.4	13.1±1.5
Rhinitis	46 (58.2%)	40 (58.0%)
Sinusitis	38 (48.1%)	31 (44.9%)
Blood eosinophils (%)	3.51±0.34	4.82±0.56†
Blood eosinophils >450/mm^3	9 (11.3%)	16 (23.2%)
Serum total IgE >100 IU/mL	51 (63.8%)	34 (49.3%)
Atopy‡	54 (69.2%)	35 (51.5%)
House dust mite sensitization*	47 (60.3%)	20 (29.9%)
Baseline		
FEV1 (% predicted)	63.2±1.3	67.4±1.3†
FEV1 (% personal best)	88.5±1.0	84.8±1.6†
FEV1/FVC (% predicted)	81.7±1.4	84.8±1.1
Min%Max PEF	83.7±2.9	84.0±2.9
Asthma activity grade	1.35±0.14	1.65±0.19
Follow-up		
FEV1 (% personal best)	89.7±0.9	84.7±1.8†
FEV1/FVC (% predicted)	80.9±1.5	87.9±1.2§‖
Min%Max PEF	87.3±2.3¶	88.5±2.1¶
Asthma activity grade	1.14±0.12	1.65±0.20†
ΔFEV1 (mL)	54.0±21.9	83.3±36.8
ΔFEV1 (%)	2.97±1.27	5.11±2.38
BCG response		
Good	15 (18.8%)	14 (20.3%)
Fair	12 (15.0%)	13 (18.8%)
Poor	53 (66.3%)	42 (60.9%)

*$P<0.001$, ‡$P<0.05$, chi-squared test.
†$P<0.05$, §$P<0.001$, compared with males.
‖$P<0.01$, ¶$P<0.05$, compared with baseline.
Asthma activity was graded using one point for every 6% fall in FEV1 from the personal best value or for every 10% fall of minimal to maximal peak expiratory flow (Min%Max PEF).

Table 2. Clinical characteristics of subjects with asthma classified according to asthma activity and their response to BCG vaccination

	Activity (+) (n=109)	Activity (-) (n=40)
Gender, female	53 (48.6%)	16 (40.0%)
Age (yr)	55.8±1.4*	61.7±1.6
Smoking (pack-years)	20.6±2.9†	31.8±5.0
Duration of asthma (yr)	11.6±1.0	15.5±2.5
Rhinitis	61 (56.0%)	25 (64.1%)
Sinusitis	47 (43.1%)	22 (56.4%)
Blood eosinophils (%)	4.31±0.37	3.57±0.66
Blood eosinophils >450/mm^3	21 (19.3%)	4 (10.0%)
Serum total IgE >100 IU/mL	61 (56.0%)	24 (60.0%)
Atopy	62 (58.5%)	27 (67.5%)
House dust mite sensitization	48 (45.7%)	19 (47.5%)
Baseline		
FEV1 (% predicted)	64.4±1.1	67.2±1.8
FEV1 (% personal best)	83.5±1.0‡	96.9±0.5
FEV1/FVC (% predicted)	83.3±1.0	82.6±1.9
Min%Max PEF	77.2±2.2‡	95.1±0.8
Follow-up		
FEV1 (% personal best)	85.8±1.2‡§	92.4±1.0‖
FEV1/FVC (% predicted)	84.1±1.1	83.3±2.4
Min%Max PEF	85.4±2.0*‖	92.9±1.3
ΔFEV1 (mL)	93.9±22.5†	-4.0±45.3
ΔFEV1 (%)	5.52±1.47†	-0.30±2.61
BCG response¶		
Good	24 (22.0%)	5 (12.5%)
Fair	22 (20.2%)	3 (7.5%)
Poor	63 (57.8%)	32 (80.0%)

*$P<0.01$, †$P<0.05$, ‡$P<0.001$, compared with activity (-).
§$P<0.05$, ‖P <0.001; compared with baseline.
¶$P<0.05$, chi-squared test.
Asthma activity was defined as ≥1 point and graded using 1 point for every 6% fall in FEV1 from the personal best value or every 10% fall of minimal to maximal peak expiratory flow (Min%Max PEF).

creased after BCG vaccination; however, in those with inactive asthma, FEV1 % personal best was significantly decreased.

There was an inverse correlation between age and degree of FEV1 improvement after BCG treatment (ΔFEV1), but there was a positive correlation between peripheral blood eosinophil % and ΔFEV1 (Fig. 1). FEV1 % personal best, but not FEV1 % predicted, was significantly related to ΔFEV1 (Fig. 2).

The rate of good/fair responses was significantly higher in subjects with eosinophilia (≥450/mm^3)[20] (40.0%/28.0% vs. 15.3%/14.5%, χ^2=13.5; P=0.001). Good or fair response rates each occurred in 21.3% of atopic, but only 15.8% and 10.5% of non-atopic, subjects. The 42.7% good+fair response rate in atopic subjects was significantly higher than the 26.3% rate in non-atopic subjects (P=0.045; Fig. 3). Although atopic and non-atopic male subjects did not differ in their good+fair response rate (33.3% each), atopic female subjects had a significantly higher rate of good+fair responses (57.1% vs. 21.2%; P<0.01). Among atopic subjects, the good+fair response rate in females was significantly higher than in males (57.1% vs. 33.3%; P=0.027). When the analysis was limited to subjects younger than 50 years old, 10/11 (90.9%) females showed a good or fair response compared with 4/10 (40.0%) males (P=0.024; Fig. 3).

Good responders to BCG vaccination were significantly younger and had a significantly higher blood eosinophil % than poor responders (Table 3). The distribution of subjects with eosinophilia differed significantly depending upon responses to BCG vaccination, but the distribution of subjects with positive reactions to atopy markers did not. FEV1 % predicted was significantly lower in good than that in fair responders, and FEV1 % personal best was significantly lower in good than that in fair/poor responders. The FEV1/FVC ratio was significantly higher in good than in poor responders after BCG vaccination. The grade of asthma activity before BCG vaccination was significantly higher in good than in poor responders, but this was reversed after BCG vaccination. Compared with baseline, lung function and asthma activity grade were improved after BCG vaccination in good/fair responders, with the exception of Min%/Max in fair responders. However, FEV1 % personal best was significantly decreased and asthma activity grade increased significantly after BCG vaccination in poor responders.

Crude odds ratios for good/fair responses were significant in terms of age, blood eosinophil %, atopy, FEV1 % personal best, and asthma activity grade (Table 4). In males, blood eosinophil %, FEV1 % personal best, and asthma activity grade were significant and, in females, age, blood eosinophil %, atopy and FEV1 % personal best were significant. No adjusted odds ratio was

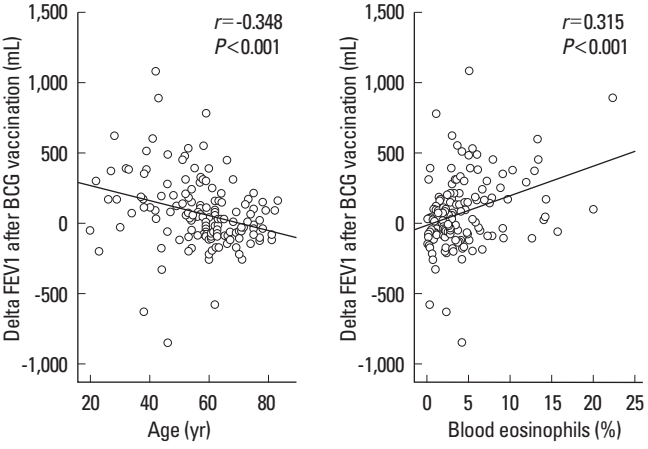

Fig. 1. Relationship between the change in forced expiratory volume in 1 sec (FEV1) after BCG vaccination and age (left panel) or peripheral blood eosinophil % (right panel).

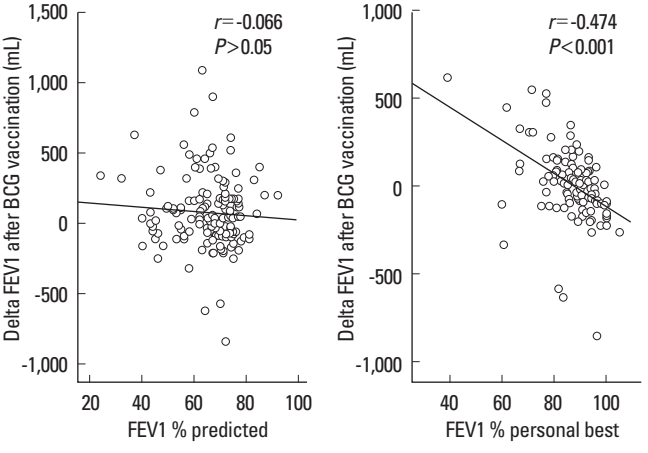

Fig. 2. Relationship between the change in forced expiratory volume in 1 sec (FEV1) after BCG vaccination and baseline FEV1 expressed as % predicted value (left panel) or % personal best value (right panel).

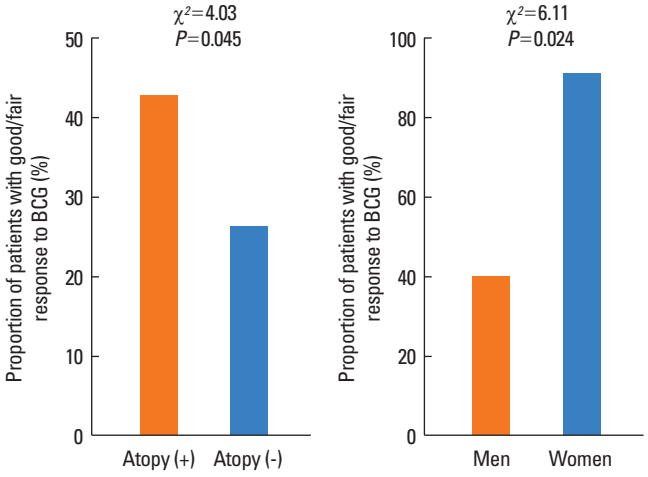

Fig. 3. Proportion of subjects who showed a good or fair response to BCG vaccination to total subjects among asthma patients with or without atopy (left panel) and in ≤50 year-old males and females with atopic asthma (right panel). Good or fair response: Δforced expiratory volume in 1 sec (FEV1) ≥6% and 100 mL or Δpeak expiratory flow (PEF) ≥10% and 30 L/min; atopy: wheal size of allergen >25% of that of histamine or allergen-specific IgE ≥0.35 kU/L (UniCAP).

Table 3. Clinical characteristics of subjects with asthma classified according to their response to BCG vaccination

	Good responder (n=29)	Fair responder (n=25)	Poor responder (n=95)
Gender, female	14 (48.3%)	13 (52.0%)	42 (44.2%)
Age (yr)	49.3±2.2*	55.2±3.1	60.4±1.3
Smoker	13 (44.8%)	8 (33.3%)	41 (43.2%)
Duration of asthma (yr)	12.1±1.9	11.6±2.2	13.0±1.4
Rhinitis	14 (48.3%)	17 (68.0%)	55 (58.5%)
Sinusitis	10 (34.5%)	15 (60.0%)	44 (46.8%)
Blood eosinophils (%)	5.96±0.88†	5.05±0.71	3.30±0.36
Blood eosinophils >450/mm^3‡	10 (34.5%)	7 (28.0%)	8 (8.4%)
Serum total IgE >100 IU/mL	17 (58.6%)	17 (68.0%)	51 (53.7%)
Atopy	19 (67.9%)	19 (76.0%)	51 (54.8%)
House dust mite sensitization	10 (35.7%)	12 (48.0%)	45 (48.6%)
Baseline			
FEV1 (% predicted)	61.3±2.5§	69.3±2.2	65.2±1.1
FEV1 (% personal best)	73.9±3.8*‖	85.1±1.3	89.1±0.9
FEV1/FVC (% predicted)	78.9±2.3	84.3±1.9	84.0±1.1
Min%Max PEF	82.9±2.7	81.7±4.3	88.8±4.0
Asthma activity grade	2.14±0.32†	1.68±0.23	1.24±0.14
Follow-up			
FEV1 (% personal best)	92.7±3.0¶	92.2±1.3¶	85.9±1.1¶
FEV1/FVC (% predicted)	86.7±2.0**‡‡	86.7±2.5‡‡	82.0±1.4
Min%Max PEF	88.5±2.2‡‡	85.6±3.1	90.6±2.2
Asthma activity grade	0.83±0.22¶**	0.88±0.18¶**	1.67±0.15¶

*P<0.001, †P<0.01, **P<0.05, compared with poor responder.
‡P<0.05, chi-squared test.
§P<0.05, ‖P<0.01, compared with fair responder.
¶P<0.001, ††P<0.05, ‡‡P<0.01, compared with baseline.
Asthma activity was graded using one point for every 6% fall in FEV1 from the personal best value or every 10% fall of minimal to maximal peak expiratory flow (Min%Max PEF).

Table 4. Crude odds ratios (95% confidence interval) for a good/fair response to BCG vaccination

	Total	Male	Female
Gender, female	1.262 (0.646-2.465)		
Age (yr)	0.952 (0.926-0.979)*	0.981 (0.946-1.019)	0.921 (0.880-0.964)*
Smoker	0.864 (0.436-1.713)		
Blood eosinophils (%)	1.165 (1.058-1.284)†	1.184 (1.006-1.394)‡	1.152 (1.020-1.302)‡
Serum total IgE (IU/mL)	1.255 (0.705-2.233)		
Atopy	2.086 (1.012-4.303)‡	1.000 (0.361-2.773)	4.952 (1.699-14.44)†
Baseline FEV1 (% best)	0.908 (0.862-0.956)*	0.861 (0.788-0.941)*	0.931 (0.875-0.991)‡
Asthma activity grade	1.415 (1.104-1.814)†	2.144 (1.363-3.373)*	1.110 (0.821-1.502)

*P<0.001, †P<0.01, ‡P<0.05.

significant in total or for either gender. Similar results were obtained in subjects with active asthma; however, in females, age, blood eosinophil % and atopy were significant, and adjusted odds ratios showed that age and atopy were significant.

DISCUSSION

In this study, BCG vaccination was effective in young female subjects. In elderly patients, lymphocyte proliferation and delayed hypersensitivity by antigen stimulation are decreased,[21] which may lead to a decreased effect of BCG vaccination. Our

previous studies showed that FEV1 was increased, from 2.16 L to 2.43 L (12.5%) in middle-aged subjects (mean age 44.9 years)[7] and from 2.14 L to 2.38 L (11.2%) in those with a mean age of 54.2 years[16] 1 month after BCG vaccination. However, in this study, FEV1 was increased by only 5.5% in subjects with active asthma, which may be explained by their greater age (55.8 years). In elderly subjects, β-adrenergic receptor affinity, adenyl cyclase activity[22] and the number of glucocorticoid receptors[23] are reduced. Since treatment outcomes were analyzed after add-on BCG vaccination without placebo controls, concomitant therapy including steroids and its age-dependency may have impacted the data.

BCG vaccination was effective in atopic allergy associated with Th2 reactions. BCG vaccination suppresses the development of asthma through Th1/Treg cell reactions in animal models of atopic asthma induced by sensitization and provocation with ovalbumin[2-6,10,12,24] or *D. farina*,[24] as well as IL-5 production in PBMCs of atopic patients with asthma.[11] The relatively high prevalence of atopic asthma in females, particularly those of child-bearing age, is likely due to elevated female sex hormone levels.[25] Thus, BCG vaccination is thought to be effective in female patients with active atopic asthma.

Three-quarters of male, but only 5.8% of female, subjects were past or current smokers. This may have affected both clinical characteristics and the therapeutic effect of BCG vaccination. The fact that FEV1 and FEV1/FVC ratio expressed as % predicted were lower in males may be explained by smoking-related irreversible airflow obstruction. The inactive asthma group contained a higher proportion of smokers, and male subjects showed a higher FEV1 % personal best and a lower asthma activity. It is thus conceivable that male subjects received BCG vaccination due not to inadequate control of asthma, but to lower lung function induced by smoking. Despite the higher house dust mite sensitization rate and the higher prevalence of atopy in males, the poor effect of BCG vaccination on their FEV1/FVC ratio in males, compared with females, may be attributed to smoking. Because smoking increases total serum IgE, facilitates allergic sensitization and induces allergic diseases,[26,27] males show a higher rate of sensitization to house dust mites and prevalence of atopy. However, Lazarus et al.[13] demonstrated that smoking decreased the effect of steroids. Smoking down-regulates histone deacetylase activity, increases tumor necrosis factor-α production, decreases the proportion of glucocorticoid isoform-α (GR-α) relative to GR-β, and promotes the synthesis of cysteinyl leukotrienes.[13] The therapeutic effect of BCG vaccination may also be influenced through such mechanisms.

In males, the factors that affected the therapeutic effect of BCG vaccination included blood eosinophil %, FEV1 and asthma activity, but not age or atopic allergy. This may be because many male subjects, even though they were enrolled due to their low lung function, might have only airway remodeling remnants after suppression of eosinophilic airway inflammation because those who had received asthma treatments for ≥6 months and had been stable for ≥1 month, were selected. Alternatively, such subjects may have been suffering smoking-related irreversible airflow obstruction and low asthma activity. Thus, in such individuals only a small proportion of disease components were reversible. Even if subjects were more highly sensitized to allergens, the effect of BCG may have been lower in those whose condition was in a drug-induced stable state. However, the therapeutic effect of BCG vaccination was significantly related to asthma activity grade and markers for asthma activity, such as blood eosinophil % and personal best FEV1 %, but not to predicted FEV1 %. When only male subjects with active asthma were included, the above markers for asthma activity, but not age and atopy, were significant factors affecting the likelihood of a good/fair response to BCG vaccination. Because the effect of smoking can obscure the influence of age and atopy, further studies with non-smokers are needed to evaluate the latter. Asthma occurs more frequently and the response to montelukast is better in males during childhood, but this trend is reversed after adolescence by the effects of female sex hormones.[28] This was interpreted as indicating that asthma was more active during childhood in boys, but in girls after adolescence. Similarly, in this study BCG vaccination was more effective in subjects with active asthma.

Because vaccination is more effective in female mice,[12] it seems likely that BCG vaccination may also be more effective in female subjects. In this study, female subjects showed a higher, but non-significant, trend towards good/fair responses. Although the therapeutic effect of BCG vaccination was related to the prevalence of atopy and house dust mite sensitization in females, the lower prevalence of atopy and house dust mite sensitization rate in female subjects might have caused the lack of statistical significance. When only atopic patients were included, good/fair responses were more frequent in females than in males (57.1 vs. 33.3%). Because atopic asthma occurs more frequently in females of child-bearing age,[25] when only female subjects aged <50 years were included, 10/11 (90.9%) versus 40.0% of males responded to BCG vaccination. The one female who did not respond to BCG vaccination was 46 years old and may have been going through menopause. Thus, BCG vaccination seems to be effective for treatment of atopic asthma in premenopausal females. Males have a higher serum level of dehydroepiandrosterone sulfate (DHEA-S), and an increased IFN-γ:IL-5 ratio induced by DHEA administration is inversely correlated with DHEA-S concentration.[29] Thus, it is conceivable that males may adapt to increased DHEA and other androgen concentrations, which decreases the Th1-induced therapeutic effect of BCG vaccination.

FEV1 was significantly decreased in subjects with inactive asthma after BCG vaccination, and asthma activity grade increased in poor responders. Even without BCG vaccination,

subjects with stable asthma who had no decrease in personal best FEV1 % may have decreased lung function after 1 month because FEV1 could not increase above the personal best value. Similarly, subjects with a very low grade of activity would naturally show increased activity over time. Such a phenomenon of regression towards the mean[30] in statistics may thus explain the above result. However, BCG infection induces neutrophilia,[10,31] and so we cannot exclude the possibility that it may also lead to aggravation of asthma even if only in a transient fashion.

The grade of asthma activity used in this study was arbitrarily defined and so its validity needed to be confirmed. Because relatively few subjects responded to BCG vaccination, we evaluated therapeutic responses by changes in the 50% values of reversibility of airflow obstruction.[14] Such a small change may occur in the absence of any treatment. Thus, further work with a larger sample size and placebo control is needed. A clinically significant wheal reaction is typically defined as >3+,[32] but we defined a positive reaction as >1+ (25% of the wheal reaction produced by histamine) because the response tended to be lower due to the higher mean age of subjects. Thus, further investigations using different criteria for skin reactions are warranted.

Taken together, age and atopy were the most important factors influencing the therapeutic effect of BCG vaccination on asthma in females and BCG vaccination was most effective in pre-menopausal females with atopic asthma. In men, asthma activity markers, such as peripheral blood eosinophil % and personal best FEV1 %, were the major factors, and the high proportion of smokers among male subjects influenced the outcomes. Thus, physicians should consider gender, age, atopy, asthma activity, and smoking to achieve better outcomes from using BCG vaccination as a treatment for asthma.

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