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BCG Infection during Pre-Sensitization or Even Post-Sensitization Inhibits Airway Sensitivity in an Animal Model of Allergic Asthma

The objective of this study is to investigate whether BCG infection before, during or after sensitization suppresses allergen-induced airway hyperresponsiveness and eosinophilic inflammation in allergic asthma rats, and to determine the required dose of BCG to induce such an inhibition. Eighty-seven Sprague-Dawley (SD) rats were sensitized and provoked with ovalbumin (OA). A pretreatment of 6×10^4 or 6×10^5 colony forming units (CFUs) of BCG or saline was done at four different times: 3 days before sensitization, at sensitization, 3 days before provocation, or at provocation. The assessment of tracheal smooth muscle (TSM) responsiveness to electrical field stimulation or acetylcholine (ACh) and bronchoalveolar lavage (BAL) were performed 1 day after OA provocation. Doses of 6×10^4 CFUs inhibited TSM sensitivity of rats infected 3 days before sensitization or at sensitization, but not 3 days before provocation or at provocation. However, doses of 6×10^5 CFUs significantly inhibited not only the airway eosinophilia of rats infected 3 days before sensitization or at sensitization, but also the TSM sensitivity of rats infected 3 days before provocation or at provocation. In conclusion, BCG infection suppresses the development of sensitivity of airway smooth muscle and airway eosinophilic inflammation in allergic asthma rats. Furthermore, a relatively high dose of BCG infection inhibits airway sensitivity, even after allergen sensitization.

Key Words: *BCG Vaccine; Asthma; Bronchial Hyperreactivity; Eosinophilia*

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INTRODUCTION

Bronchial asthma is a chronic inflammatory disorder of the airways which causes an associated increase in the existing bronchial hyperresponsiveness to a variety of stimuli (1). A TH2 lymphocyte cytokine profile is instrumental in initiating and sustaining the inflammatory process in bronchial asthma (2, 3). $CD4^+$ T cells may be divided into TH1 and TH2 populations, which secrete different cytokines. TH1 cytokines inhibit the production of TH2 cytokines, whereas TH2 cytokines, which are important in allergic disease, inhibit the production of TH1 cytokines, accentuating the production of polarized cytokine profiles during immune responses (4).

The conversion of TH2 into TH1 cells is thought to be desirable in patients with allergic diseases, in particular bronchial asthma. Recent reports, supported by other studies (5-7), show that viral (8) or bacterial infection (9) may inhibit allergic diseases by producing cytokine profiles biased toward TH1 type. *Mycobacterium* species, including bacillus Calmette-Guerin (BCG) used in the vaccine to prevent tuberculosis, are known to be potent inducers of a TH1-type response (10-14). BCG vaccination was recommended at 4 weeks of life to all newborns in Korea (15).

We developed an animal model of allergic asthma in which the sensitivities of airway smooth muscle to cholinergic stimuli increased in Sprague-Dawley (SD) rats sensitized with 10 μg ovalbumin (OA) and provoked with 5% OA aerosols (16). The aim of this study is threefold: 1) to investigate whether BCG infection suppresses airway hyperresponsiveness and eosinophilic inflammation developed in OA sensitized and provoked SD rats; 2) to determine the required dose of BCG to induce such an inhibition; and 3) to demonstrate whether BCG infection even after allergen sensitization inhibits the development of allergic asthma in the animal model.

MATERIALS AND METHODS

Experimental rats

Eighty-seven male SD rats (Sam-Yook animal com-

Table 1. The characteristics of each group classified by the time points and dose of BCG infections and the procedures performed for each group

Group	Time points of BCG infection	BCG dose (CFUs)	N (87)	10 μg-OA sensitization	5%-OA provocation	Muscle study	BAL study
Control	–	–	16	+	+	+	+
BCG-I	3 days before sensitization	6×10^4	8	+	+	+	+
		6×10^5	9	+	+	+	+
BCG-II	at sensitization	6×10^4	10	+	+	+	+
		6×10^5	9	+	+	+	+
BCG-III	3 days before provocation	6×10^4	7	+	+	+	+
		6×10^5	10	+	+	+	+
BCG-IV	at provocation	6×10^4	8	+	+	+	+
		6×10^5	10	+	+	+	+

BCG, bacillus Calmette-Guerin; CFUs, colony forming units; N, the number of rats; OA, ovalbumin; BAL, bronchoalveolar lavage

pany Ltd., Korea) were used in this study. They were specific pathogen-free rats that were raised on standard diets in an animal care room at Chonnam National University Medical School until they reached 200 g body weight (17). They were all sensitized and provoked with OA and divided into five different groups according to the time points of BCG infection in relation to OA sensitization and provocation: 16 controls, 17 rats infected with BCG 3 days before OA sensitization, 19 rats infected with BCG at OA sensitization, 17 rats infected with BCG 3 days before OA provocation, or 18 rats infected with BCG at OA provocation. The two different doses of 6×10^4 and 6×10^5 colony forming units (CFUs) of BCG were used in each group (Table 1).

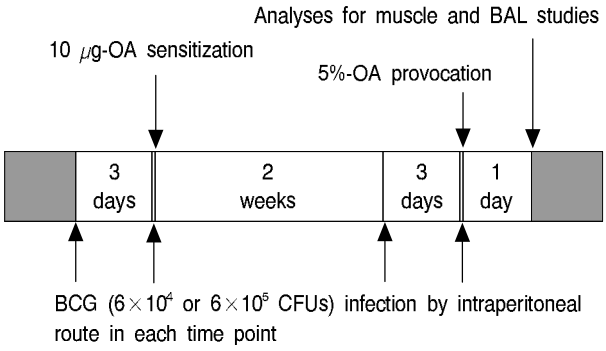

Fig. 1. Schematic representation of the time course of ovalbumin (OA) sensitization and provocation, and BCG infection in this experimental study.

Protocol

Fig. 1 provided a schematic diagram of the time course of OA sensitization and provocation, and BCG infection.

OA sensitization and provocation

All 87 SD rats were actively sensitized with a subcutaneous injection of 10 μg OA (Grade III, Sigma) together with 200 mg $Al(OH)_3$ and 1 mL killed *Pertussis* vaccine (1×10^{10}/mL, Korea Green Cross Corporation). Two weeks later, they were provoked with 5% OA aerosols using Pari Boy nebulizer (output=0.42 g/min, mass median aerodynamic diameters=4.8 μm, pressure=0.75 bar) in an exposure chamber (length=56 cm, width =44 cm, height=46 cm) developed in our laboratory.

BCG infection

Seventy-one non-control rats were infected intraperitonealy with live attenuated BCG (6×10^4 and 6×10^5 CFUs, Pasteur Merieux, France) at the different time points (Table 1).

Bronchoalveolar lavage (BAL)

The rats were euthanized by an intraperitoneal injection of 5 mg/kg thiopental sodium and exsanguinated through the inferior vena cava 24 hr after the provocation with OA aerosols. The trachea was canulated with PE-20 polyethylene tube and BAL was performed by 5 lavages with 4 mL physiologic saline (4℃). The recovered fluid was immediately centrifuged at 1,500 rpm for 10 min at 4℃ (VS-6000, Vision, Korea). The cell pellet was resuspended with 0.5 mL PBS (pH=7.4). Total cells were counted under light microscopy (100×) using hemocytometer (Neubauer chamber) after mixing the 50 μL cell pellet solution with 950 μL acetic acid (3%). The remaining cell pellet solution was diluted with PBS to make cell counts 1×10^6/mL. Cytospins were prepared for each sample by centrifuge of 400 μL cell pellet solution at 700 rpm for 5 min at 4℃ (CF-120, Sakura, Japan). Two slides for each sample were obtained. After fixations, each slide was stained with Diff-Quik. Differential cell counts of 500 cells in each slide were performed under light microscopy (400×) and mean cell counts of the two slides were used for statistical analysis. The cells were

classified as either neutrophils, eosinophils, lymphocytes, or macrophages by morphologic criteria.

Assessment of airway smooth muscle responsiveness

After BAL was performed, the trachea was immediately removed and was placed in oxygenated fresh Krebs-Henseleit (K-H) solution (115.5 NaCl, 4.16 KCl, 2.5 $CaCl_2$, 1.16 $MgSO_4$, 1.6 NaH_2PO_4, 21.9 $NaHCO_3$, and 11.1 mM glucose). The trachea was trimmed free of fat and connective tissue and cut into 4 transverse pieces, each containing 4 to 5 cartilagenous rings. The epithelium was left intact. Tracheal ring segments were mounted vertically using platinum hooks inserted through the lumen in 10 mL organ baths containing K-H solution (pH=7.4) bubbled with 95% O_2 and 5% CO_2 at 37℃. The upper hook was fastened to a force transducer (Grass FT03) using silk thread (Ethicon 4-0). Tissues were equilibrated for one and a half hr with washing at least every 20 min under a resting tension of 0.75 g. In a preliminary study, the optimal tension for the contractile response of tracheal ring segment of SD rats was found to be 0.75 g. Isometric contractile responses were measured with force transducer and were recorded on a polygraph (Grass 7 series).

During the equilibrium period, the maximal response to electrical field stimulation (EFS) was measured. The lower platinum hook served as one of the poles of the EFS source and the other pole for EFS was not placed in physical contact with the segment; the two poles were 1 cm apart. Pulses were delivered for 10 seconds from an electrical stimulator (Dual Impedance Research Stimulator, Harvard) with a voltage of 50 V. In the preliminary study, the optimal voltage was 50. With a pulse duration of 0.5 msec, 0.5, 1, 2, 5, 10, 20, 50 and 100 Hz were delivered with one and a half min intervals in progressively increasing pulses. After EFS-induced responses, the tissue was washed to regain the baseline tension and the maximal responses to a high KCl K-H solution (120 mM KCl in equimolar replacement for NaCl) were measured. The tissue was then washed repeatedly to regain the baseline tension and soaked for 30 min. Finally, the cumulative dose-response relationships to acetylcholine (ACh: 0.1, 0.3, 1, 3, 10, 30, 100, 300, 1000 μM) were measured. The maximum active tensions developed were recorded for each concentration. The highest active tension developed for each dose of the used stimulant was considered as the maximal response to the stimulant. After the experiment, the segments were air-dried and weighed.

The responses were expressed as a gram force/gram tissue (g/g) and a percentage of the maximum response to the high KCl (% KCl) for each tissue. The frequency of EFS ($EFS\text{-}EC_{50}$) and the cumulative dose of ACh ($ACh\text{-}EC_{50}$) causing 50% of the maximal responses were calculated.

Statistical analysis

Tracheal ring segments which were unresponsive to KCl, EFS, or ACh were excluded from data analysis because they were considered as damaged specimens. The mean value of $EFS\text{-}EC_{50}$ (Hz) or $ACh\text{-}EC_{50}$ (μM) obtained from each tracheal ring segment in each rat was used in statistical analyses. Results were expressed as means±SEM values. Student's t test or Mann-Whitney U test was used to determine the level of difference between groups. Pearson's correlation was used to examine the relationships between airway muscle sensitivities and inflammatory cells of BAL fluid. A p value of less than 0.05 was considered significant.

RESULTS

Decreased sensitivity of airway smooth muscle in BCG-infected rats

When OA sensitized and provoked rats were infected with 6×10^4 CFUs of BCG, the sensitivities of tracheal smooth muscle (TSM) to EFS ($EFS\text{-}EC_{50}$) significantly decreased in rats infected 3 days before sensitization (13.92±0.88 Hz, $p<0.05$), at sensitization (14.06±1.16 Hz, $p<0.05$), or 3 days before provocation (13.40±0.39 Hz, $p<0.01$) compared with controls (11.38±0.43 Hz). However, $EFS\text{-}EC_{50}$ in rats infected at provocation did not significantly differ from the controls. The sensitivities of tracheal smooth muscle (TSM) to ACh ($ACh\text{-}EC_{50}$) significantly decreased in rats infected 3 days before sensitization (21.72±2.47 μM, $p<0.01$) or at sensitization (25.76±3.62 μM, $p<0.01$) compared with controls (12.65±1.22 μM). However, $ACh\text{-}EC_{50}$ in rats infected 3 days before provocation or at provocation did not significantly differ from the controls (Fig. 2). The maximal contractile responses (g/g) of TSM to EFS significantly decreased only in those rats infected 3 days before sensitization and those to ACh significantly decreased only in rats infected 3 days before sensitization or at sensitization ($p<0.05$, data not shown). The maximal responses (% KCl) of TSM to EFS or ACh in rats infected at any time did not decrease compared with controls (data not shown).

When OA sensitized and provoked rats were infected with 6×10^5 CFUs of BCG, the sensitivities of TSM to EFS significantly decreased in rats infected 3 days before sensitization (13.50±0.91 Hz, $p<0.05$), 3 days before provocation (16.94±1.15 Hz, $p<0.001$), or at provoca-

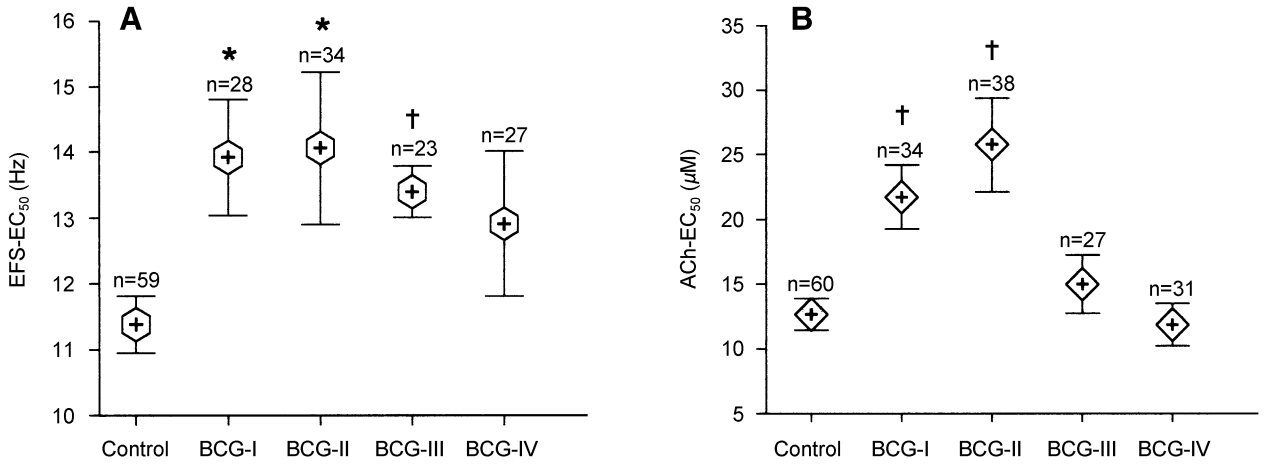

Fig. 2. The sensitivity of tracheal smooth muscle to EFS (A) and ACh (B) in controls and four different BCG (6×10^4 CFUs)-infected groups (BCG-I, BCG-II, BCG-III, and BCG-IV). N refers to the number of tracheal muscle segments obtained from each group. Data are expressed as means±SEM. *$p<0.05$, †$p<0.01$ versus controls by the Student's t-test.

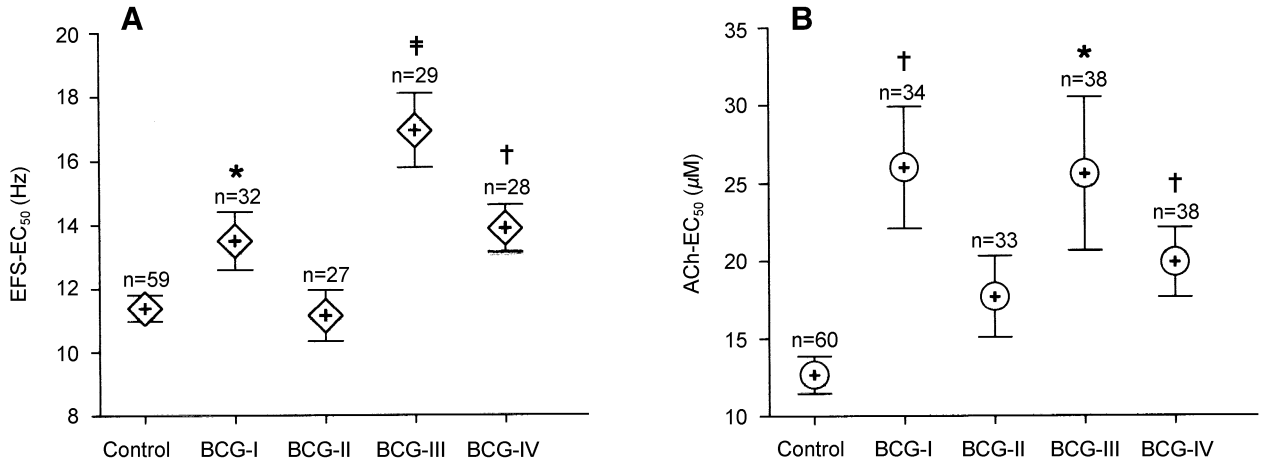

Fig. 3. The sensitivity of tracheal smooth muscle to EFS (A) and ACh (B) in controls and four different BCG (6×10^5 CFUs)-infected groups (BCG-I, BCG-II, BCG-III, and BCG-IV). N refers to the number of tracheal muscle segments obtained from each group. Data are expressed as means±SEM. *$p<0.05$, †$p<0.01$, ‡$p<0.001$ versus controls by the Student's t-test.

tion (13.87±0.74 Hz, $p<0.01$) compared with controls (11.38±0.43 Hz). The EFS-EC_{50} in rats infected at sensitization did not differ significantly from the controls. Similarly, the sensitivities of TSM to ACh significantly decreased in rats infected 3 days before sensitization (26.01±3.90 μM, $p<0.01$), 3 days before provocation (25.58±4.92 μM, $p<0.05$), or at provocation (19.89±2.23 μM, $p<0.01$) compared with controls (12.65±1.22 μM, Fig. 3). The ACh-EC_{50} in rats infected at sensitization did not differ significantly from the controls. The maximal contractile responses, expressed as either g/g or % KCl, of TSM to EFS or ACh did not significantly decrease in rats infected at any time compared with controls (data not shown).

Decreased eosinophil counts of BAL fluid in BCG-infected rats

When OA sensitized and provoked rats were infected with 6×10^4 CFUs of BCG, the mean total cell, neutrophil and lymphocyte counts of the BAL fluid did not decrease in rats infected at any time compared with controls. However, eosinophil counts of BAL fluid decreased, although not significantly, in rats infected 3 days before sensitization, at sensitization and at provocation compared with controls (Fig. 4).

When OA sensitized and provoked rats were infected with 6×10^5 CFUs of BCG, the mean total cell and neutrophil counts of BAL fluid did not significantly decrease in rats infected at any time compared with controls. Lymphocyte counts of BAL fluid significantly increased in rats infected 3 days before provocation compared with controls. However, eosinophil counts of BAL fluid significantly decreased in rats infected 3 days before sensitization ($0.86\pm0.63\times10^3/\mu L$, $p<0.01$) or at sensitization

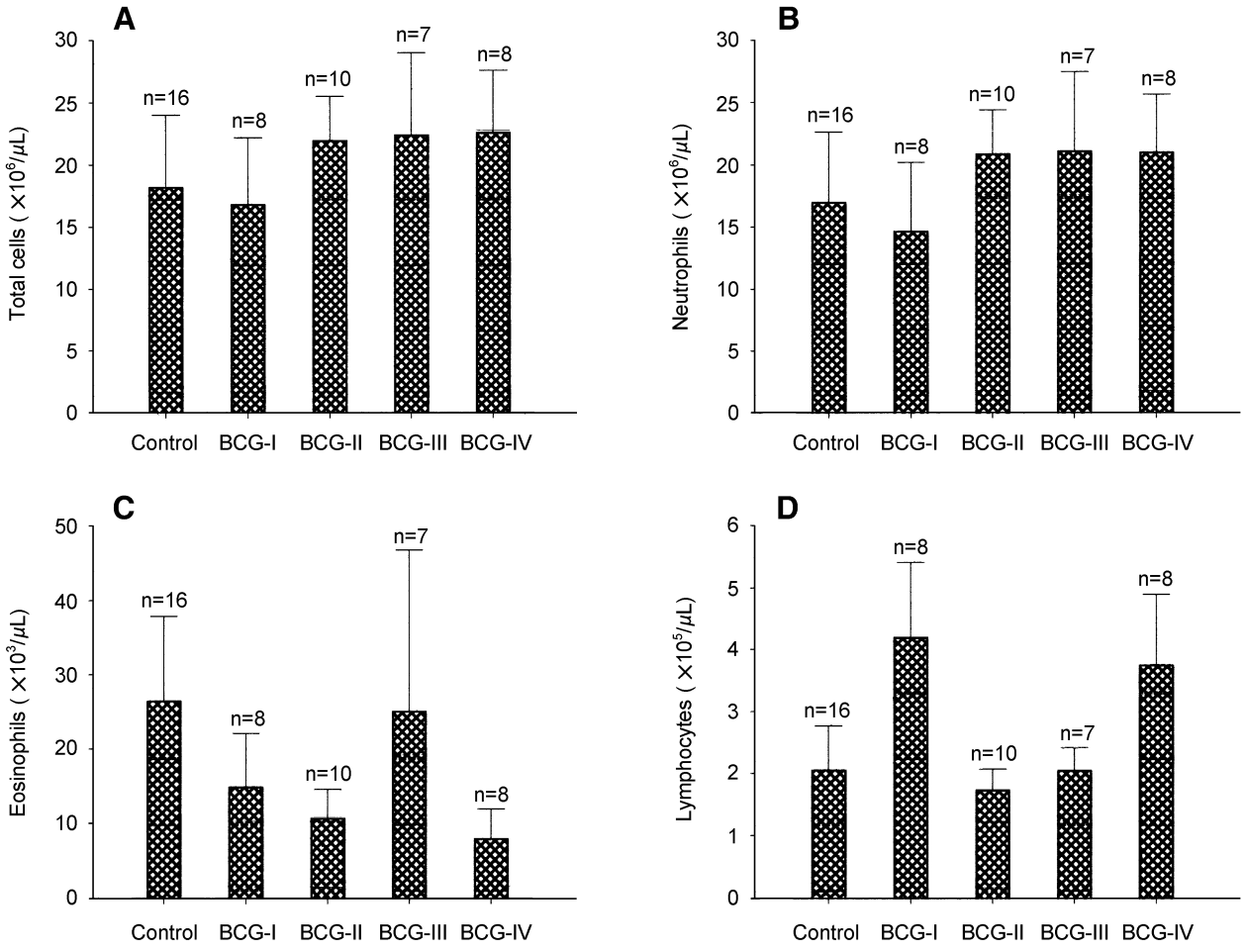

Fig. 4. Numbers of total cells (A), neutrophils (B), eosinophils (C) and lymphocytes (D) in BAL fluid obtained from controls and four different BCG (6×10^4 CFUs)-infected groups (BCG-I, BCG-II, BCG-III, and BCG-IV). N refers to the number of rats in each group. Data are expressed as means±SEM. Comparisons between each BCG-infected group and control were performed by the Mann-Whitney U-test. No statistical significance was observed.

($3.86\pm3.36\times10^3/\mu L$, $p<0.01$), but not in rats infected 3 days before provocation or at provocation, compared with controls ($26.42\pm11.48\times10^3/\mu L$, Fig. 5). In controls and in rats infected with 6×10^5 CFUs of BCG, eosinophil counts of BAL fluid significantly correlated with the sensitivities of TSM to ACh (r=-0.236, n=53, $p=0.04$).

DISCUSSION

This study demonstrates the sensitivity of airway smooth muscle to cholinergic stimuli, such as exogenous ACh or endogenous ACh which are released from cholinergic nerves by EFS, and eosinophils in BAL fluid are significantly reduced in OA sensitized and provoked rats infected with BCG. Although this study does not show the direct evidence that OA sensitization and provocation can induce allergic asthmatic responses in SD rats, our previous study demonstrated that airway muscle sensitivity and airway eosinophilila significantly increased in OA sensitized and provoked rats compared with non-sensitized and non-provoked control rats. In addition, early asthmatic response can be observed in 77.8% of 10 μg OA-sensitized and 5% OA-provoked rats (16).

Our results from this study suggest that BCG infection may hinder the development of bronchial asthma in SD rats. The most likely explanation for the results can be that TH1 cytokines produced during an active BCG infection may block the expansion of TH2 cells, and thus reduce airway eosinophils and sensitivities of airway smooth muscle to cholinergic stimuli in this animal model. Our observations are supported by recently published studies showing that BCG infection established a TH1 type immune response and then hindered allergic sensitization, allergen-induced airway eosinophilia and the development of increased airway reactivity in an animal model (18, 19). In the study by Herz et al. (18), BCG immunization in OA-sensitized mice resulted in: 1)

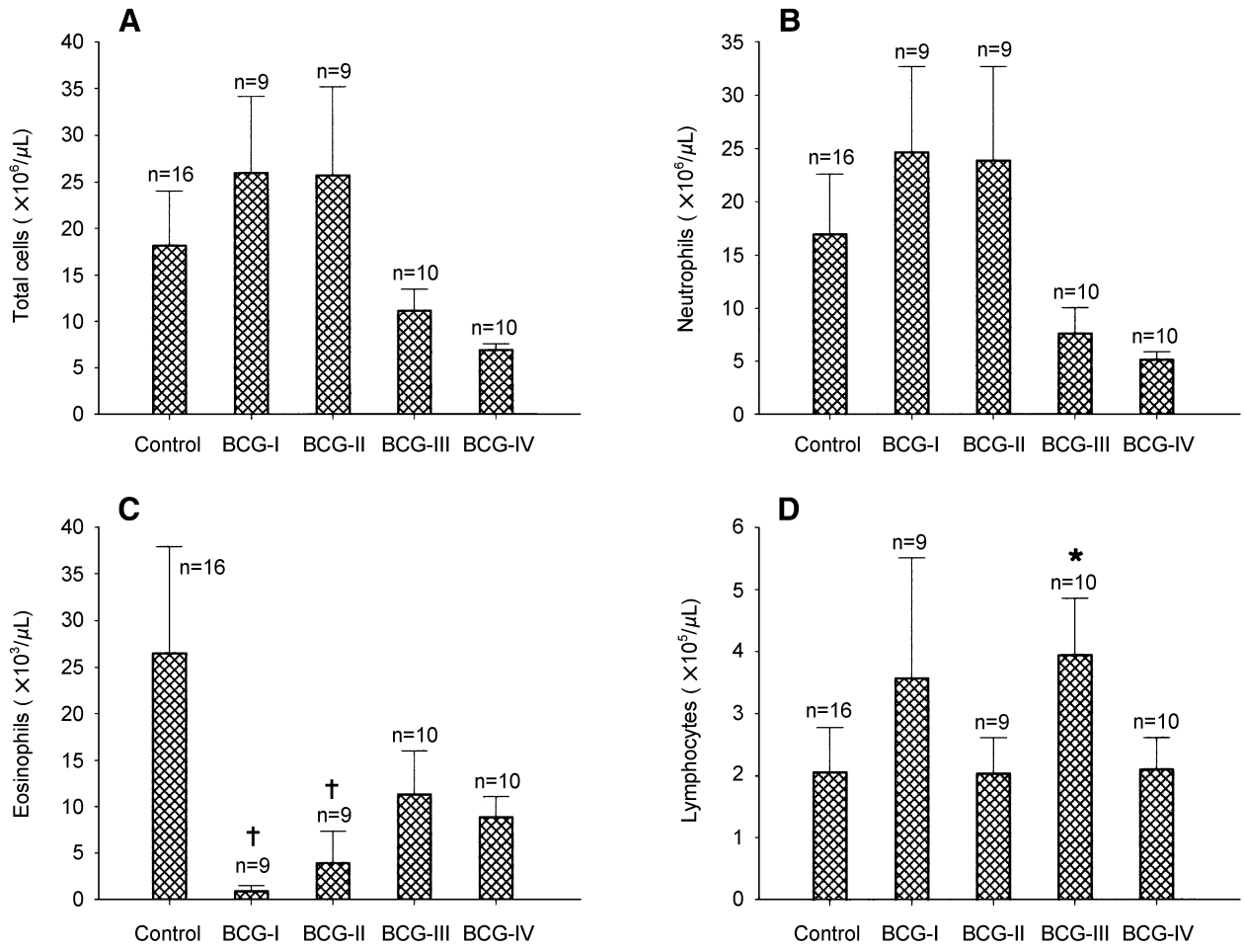

Fig. 5. Numbers of total cells (A), neutrophils (B), eosinophils (C) and lymphocytes (D) in BAL fluid obtained from controls and four different BCG (6×10^5 CFUs)-infected groups (BCG-I, BCG-II, BCG-III, and BCG-IV). N refers to the number of rats in each group. Data are expressed as means±SEM. *$p<0.05$, †$p<0.01$ versus controls by the Mann-Whitney U-test.

increased interferon (IFN)-γ and decreased IL-4 production by splenic mononuclear cells; 2) decreased OA-specific IgE and IgG1 serum antibody titers; and 3) reduced IL-4 and IL-5 levels in BAL fluids. Erb et al. (19) showed that BCG-induced inhibition of airway eosinophilia was strongly reduced in IFN-γ receptor-deficient mice, indicating that BCG-induced inhibition was mediated by IFN-γ. Cytokine measurements are needed to confirm that the suppressive effects of BCG were secondary to a TH1 induction.

To explore the effects of the BCG dose or the time point of BCG infection on airway hypersensitivities and eosinophilic inflammation, the experimental rats were infected intraperitoneally with two different doses of BCG (6×10^4 or 6×10^5 CFUs) at four different times: 3 days before OA sensitization; at OA sensitization; 3 days before OA provocation; or at OA provocation. Doses of 6×10^4 CFUs of BCG, although they did not significantly suppress the airway eosinophilia of rats infected at any time, did inhibit airway muscle sensitivities of rats infected 3 days before sensitization or at sensitization, but not 3 days before provocation or at provocation. However, doses of 6×10^5 CFUs of BCG significantly inhibited not only the airway eosinophilia of rats infected 3 days before sensitization or at sensitization, but also the airway sensitivities of rats infected 3 days before provocation or at provocation. These results indicate that a relatively high dose of BCG may reduce the airway hyperresponsiveness in asthma rats infected not only during allergen sensitization, but also during allergen provocation; such a dose is also needed to suppress the airway eosinophilia developed in the asthma rats. Unexpectedly, the airway muscle sensitivity of rats infected at sensitization was not suppressed by doses of 6×10^5 CFUs of BCG (Fig. 3). This is possibly due to a reduction in the release of an epithelium-derived inhibitory factor (20), resulting from incidental damage of epithelium during the process of tracheal muscle preparation, which may cause the decrease in the suppressive effect of BCG on airway sensitivity.

Recently, there were a few animal studies showing the suppressive effects of BCG on allergic response, in which the different doses of BCG were used and BCG infection was done at the different time points from one another. In the study by Herz et al. (18), mice were infected intravenously with 1×10^6 CFUs of BCG 14 days before OA sensitization. Erb et al. (19) showed that BCG numbers $\geq2\times10^5$ CFUs maximally inhibited airway eosinophilia in mice infected intranasally 4 weeks before OA provocation. Furthermore, Wang and Rook (21) demonstrated that even the infection of mice with *Mycobacterium vaccae* after OA sensitization could suppress an established allergic response. Unlike the other studies, airway muscle sensitivities in our experimental rats infected with 6×10^5 CFUs of BCG at OA provocation were significantly inhibited, although muscle study was performed 1 day after BCG infection. The possible explanation for this phenomenon can be that a low IFN-γ produced at days 1-2 after BCG infection (10) may prevent the development of airway hyperresponsiveness by interfering with eosinophil degranulation (22). Infecting the experimental rats with 6×10^5 CFUs of BCG 3 days before sensitization was the most effective way to inhibit airway sensitivity and eosinophilic inflammation in this study.

Airway responsiveness in this study was represented as the sensitivity of airway smooth muscle to cholinergic stimuli, and not the maximal contractile responses. The maximal contraction of airway smooth muscle did not significantly decrease in rats infected with BCG. Our previous study demonstrated that OA sensitized and provoked SD rats had increased sensitivities, but not increased maximal contractile responses, of airway smooth muscle to cholinergic stimuli (16). There were other reports that the sensitivities of airway smooth muscle increased in rats sensitized with an allergen in an animal model (23-25).

The limitation of this study is that the experimental rats may have been infected with mostly unknown bacterial organisms during the process of our experiments, although BCG infection (19) or airway inflammation in asthma (26) may in part contribute to airway neutrophilia. However, there is no significant difference in neutrophil counts of BAL fluid between BCG-infected rats and controls, indicating that this airway neutrophilia rarely influenced our results.

We propose that BCG vaccine given at 4 wks of life for preventing tuberculosis in Korea (15) may be instrumental in hindering the development of bronchial asthma in children with atopic heredity. Furthermore, our results show that a relatively high dose BCG infection inhibits airway sensitivity even after allergen sensitization, suggesting that late BCG vaccination in children sensitized with airborne allergens may be helpful in preventing the development of airway hyperresponsiveness. However, according to recent epidemiological studies (9, 27), the suppressive effect of BCG vaccine on the development of atopic disorder in children is debatable (28). Further cautious prospective and model-based clinical experiments are needed to determine the clinical outcomes of BCG vaccine in human populations.

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REFERENCES

1. *NHLBI/WHO Workshop Report: Global strategy for asthma management and prevention. National Institutes of Health, 1995. NIH Publication No 95-3659.*
2. James DG, Kay AB. *Are you TH-1 or TH-2? Clin Exp Allergy 1995; 25: 389-90.*
3. Ricci M, Rossi O, Bertoni M, Matucci A. *The importance of TH2-like cells in the pathogenesis of airway allergic inflammation. Clin Exp Allergy 1993; 23: 360-9.*
4. Umetsu DT, DeKruyff RH. *Th1 and Th2 CD4+ cells in human allergic diseases. J Allergy Clin Immunol 1997; 100: 1-6.*
5. von Mutius E, Fritzsch C, Weiland SK, Roll G, Magnussen H. *Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison. BMJ 1992; 305: 1395-9.*
6. Braback L, Breborowicz A, Dreborg S, Knutsson A, Pieklik H, Bjorksten B. *Atopic sensitization and respiratory symptoms among Polish and Swedish school children. Clin Exp Allergy 1994; 24: 826-35.*
7. von Mutis E, Martinez FD, Fritzsch C, Nicolai T, Reitmeir P, Thiemann HH. *Skin test reactivity and number of siblings. BMJ 1994; 308: 692-5.*
8. Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, Goudiaby A. *Measles and atopy in Guinea-Bissau. Lancet 1996; 347: 1792-6.*
9. Shirakawa T, Enomoto T, Shimazu S, Hopkin J. *The inverse association between tuberculin responses and atopic disorder. Science 1997; 275: 77-9.*
10. Sander B, Skansen-Saphir U, Damm O, Hakansson L, Andersson J, Andersson U. *Sequential production of Th1 and Th2 cytokines in response to live bacillus Calmette-Guerin. Immunology 1995; 86: 512-8.*
11. Lai CK, Ho S, Chan CH, Chan J, Choy D, Leung R, Lai KN. *Cytokine gene expression profile of circulating CD4+ T cells in active pulmonary tuberculosis. Chest 1997; 111: 606-11.*
12. Gros P, Skamene E, Forget A. *Genetic control of natural resistance of Mycobacterium bovis (BCG) in mice. J Immunol*

1981; 127: 2417-21.

13. Del Prete GF, De Carli M, Mastromauro C, Biagiotti R, Macchia D, Falagiani P, Ricci M, Romagani S. *Purified protein derivative of Mycobacterium tuberculosis and excretory-secretory antigen(s) of Toxocara canis expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991; 88: 346-50.*
14. Daugelat S, Ladel CH, Kaufmann SH. *Influence of mouse strain and vaccine viability on T-cell responses induced by Mycobacterium bovis bacillus Calmette-Guerin. Infect Immun 1995; 63: 2033-40.*
15. *Expanded programme on immunization (EPI) immunization schedules in the WHO Western Pacific Region, 1995. Wkly Epidemiol Rec 1996; 71: 133-7.*
16. Koh YI, Choi IS, Jang AS, Na HJ, Lee JU, Lee HC, Lee MK. *Development of allergic asthma model using rat airway smooth muscle [in Korean]. Korean J Allergy 1997; 17: 227-49.*
17. Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council. *Guide for the care and use of laboratory animals. Washington, D.C.: National Academy Press, 1996.*
18. Herz U, Gerhold K, Gruber C, Braun A, Wahn U, Renz H, Paul K. *BCG infection suppresses allergic sensitization and development of increased airway reactivity in an animal model. J Allergy Clin Immunol 1998; 102: 867-74.*
19. Erb KJ, Holloway JW, Sobeck A, Moll H, Le Gros G. *Infection of mice with Mycobacterium bovis-Bacillus Calmette-Guerin (BCG) suppresses allergen-induced airway eosinophilia. J Exp Med 1998; 187: 561-9.*
20. Pavlovic D, Fournier M, Aubier M, Pariente R. *Epithelial vs. serosal stimulation of tracheal muscle: role of epithelium. J Appl Physiol 1989; 67: 2522-6.*
21. Wang CC, Rook GA. *Inhibition of an established allergic response to ovalbumin in BALB/c mice by killed Mycobacterium vaccae. Immunology 1998; 93: 307-13.*
22. Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. *Regulatory effect of cytokines on eosinophil degranulation. J Immunol 1990; 144: 642-6.*
23. Antonissen LA, Mitchell RW, Kroeger EA, Kepron W, Stephens NL, Bergen J. *Histamine pharmacology in airway smooth muscle from a canine model of asthma. J Pharmacol Exp Ther 1980; 213: 150-5.*
24. Wagner EM, Kleeberger SR, Spannhake EW, Adams GK III. *Increased in vitro airway responsiveness in sheep following repeated exposure to antigen in vivo. J Appl Physiol 1985; 59: 1874-8.*
25. Inoue K, Sakai Y, Homma I. *Rabbit immunization to induce experimental asthma - relationship between antigens and airway reactivity. Arerugi 1991; 40: 46-50.*
26. O'Byrne PM, Walters EH, Gold BD, Aizawa HA, Fabbri LM, Alpert SE, Nadel JA, Holtzman MJ. *Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. Am Rev Respir Dis 1984; 130: 214-9.*
27. Alm JS, Lilja G, Pershagen G, Scheynius A. *Early BCG vaccination and development of atopy. Lancet 1997; 350: 400-3.*
28. Silverman M. *BCG vaccination and atopy-unfinished business? Lancet 1997; 350: 380-1.*