

ORIGINAL RESEARCH

The effect of Bacillus Calmette-Guerin in a mouse model of allergic rhinitis

Soo Whan Kim, MD, PhD, and Sang Won Yeo, MD, PhD, Seoul, South Korea

OBJECTIVE: To develop an allergic rhinitis model via local sensitization of mice with *Dermatophagoides pteronyssinus* and to use the model to investigate the antiallergic effect of Bacillus Calmette-Guerin (BCG).

METHODS: BALB/c mice were randomized to the allergic rhinitis group, the BCG group sensitized with *D pteronyssinus* via local sensitization for 7 weeks, or the control group. The BCG group received 1×10^5 colony-forming unit/mL of BCG seven times. We evaluated symptoms, nasal mucosa eosinophilia, serum total IgE, cytokines, and eosinophilia in bronchoalveolar lavage fluid.

RESULTS: In the allergic rhinitis group, allergy symptoms ($P < 0.001$), eosinophil count ($P < 0.001$), total IgE ($P < 0.001$), inflammatory cells ($P < 0.01$), and IL-13 ($P = 0.046$) increased and interferon γ (IFN- γ) ($P < 0.001$) decreased compared with the control group. In the BCG group, allergy symptoms ($P < 0.001$), eosinophil count ($P < 0.001$), and inflammatory cells ($P < 0.01$) decreased and IFN- γ ($P = 0.031$) increased compared with the allergic rhinitis group.

CONCLUSION: In an allergic rhinitis animal model developed via local sensitization of mice with *D pteronyssinus*, BCG was shown to exhibit an antiallergic effect.

© 2007 American Academy of Otolaryngology–Head and Neck Surgery Foundation. All rights reserved.

A rapidly increasing proportion of the population suffers from allergic diseases. In developed countries, the incidence of seasonal allergic rhinitis is approximately 10%, whereas the incidence of perennial allergic rhinitis ranges from 10% to 20%. Up to 40% of children have been reported to suffer from allergic rhinitis.¹ Left untreated, allergic rhinitis poses a risk for sinusitis and may be complicated by asthma. Although medical treatment is the cornerstone of allergic rhinitis management, adverse effects are frequently associated with treatment, and recurrence of symptoms is

common after drug withdrawal. Therefore, treatment modalities with improved safety profiles are needed. Since its introduction by Noon² in 1911, immunotherapy has been widely used in the management of allergic diseases. However, the adverse systemic reactions and high cost associated with immunotherapy have limited its widespread clinical application. Bacillus Calmette-Guerin (BCG) is used as a vaccine for tuberculosis and leprosy. In addition, the agent has been reported to alleviate airway hyperresponsiveness in patients with allergic asthma by inducing the Th1 immune reaction and altering cytokine regulation.³ However, these effects have not been studied in models of allergic rhinitis.

An animal model of allergy allows the study of allergic rhinitis pathophysiology and treatment. Previous studies on allergic rhinitis have been based on models using ovalbumin;⁴ however, ovalbumin is a food allergen. In the present study, we used the house dust mite because it is the most common allergen.⁵ Previous attempts to produce allergic rhinitis models with the house dust mite have involved intranasal local sensitization after systemic sensitization.⁵ We postulated that a better model would have allergen sensitization occur in the same fashion as it does naturally.

The objectives of the present study were to develop an animal model of allergic rhinitis via intranasal local sensitization of mice with the house dust mite allergen and to use this model to investigate the antiallergic effect of BCG.

MATERIALS AND METHODS

Experimental Animals and Allergen

The experiment was performed with the approval of the Institutional Animal Care and Use Committee at the Cath-

From the Department of Otolaryngology–Head and Neck Surgery, College of Medicine, The Catholic University of Korea.

Reprint requests: Sang Won Yeo, MD, Department of Otolaryngology–Head and Neck Surgery, College of Medicine, The Catholic University of

Korea, Kangnam St. Mary's Hospital, #505 Banpodong, Seochogu, Seoul 137-701, Korea.

E-mail address: swyeo@catholic.ac.kr.

olic University of Korea. Six-week-old healthy female BALB/c mice (20–30 g) were used in this study. *Derma-tophaoides pteronyssinus* lyophilized crude body extract (Yonsei University Allergen Bank, Seoul, Korea), which was used as the allergen, was stored at 4°C and thawed with phosphate-buffered saline (PBS) before use.

Production of the Allergic Rhinitis Model

Twenty-four mice were randomized to one of three groups: control ($n = 8$), allergic rhinitis ($n = 8$), or BCG treatment ($n = 8$). In the allergic rhinitis group, 25 $\mu\text{g}/20 \mu\text{L}$ of *D pteronyssinus* were mixed in PBS and micropipetted into both nostrils of each anesthetized mouse, five times every week during the first three weeks and three times every week during the next three weeks. On the seventh week, each nasal cavity was sensitized for three consecutive days (for a total of 27 sensitizations); the mice were killed 24 hours later.

In the BCG treatment group, the mice were inhalant sensitized in the same way as in the allergic rhinitis group, but BCG (Korea Vaccines, Seoul, Korea) 1×10^5 colony-forming U/mL was injected transdermally once every week for seven weeks beginning one week before the first allergen sensitization (Fig 1). The control group received intranasal inoculation with PBS 20 μL via the same method and frequency of allergen inoculation as in the allergic rhinitis group.

Evaluation of Allergic Symptoms Induced After Allergen Inoculation

The number of sneezing and nose rubbing motions during 15 minutes after the final allergen inoculation was recorded and compared among experimental groups.

Bronchoalveolar Lavage

Using ether anesthesia, thoracotomy was performed on the mice in each group 24 hours after the final allergen inhalant inoculation. The left main bronchus was ligated, and a 22-gauge needle was inserted into the trachea. Bronchoalveolar lavage (BAL) was performed in each mouse on the right lung by repeatedly inoculating 0.4 mL of PBS followed by suctioning three times. BAL fluid was then centrifuged at 2,000 rpm for seven minutes at 4°C, and the supernatant was stored at -70°C . The precipitate was evaluated for cell count by using a hemacytometer. The slides were prepared by using a cytocentrifuge (Cytospin 2; Shan-

don, Runcorn, UK), stained with Diff-Quick (Dade Behring AG, Dürdingen, Switzerland) and evaluated for counts of macrophages, eosinophils, lymphocytes, and neutrophils by observing at least 300 cells at $\times 400$ magnification.

Nasal Mucosa Tissue Evaluation

Twenty-four hours after the final allergen inoculation, the mice were killed and decapitated. The heads were fixed in 4% paraformaldehyde for three days at 4°C, washed in running water, decalcified for three days with Calci-Clear Rapid (Diagnostics, Atlanta, GA, USA) at room temperature, dehydrated with serially concentrated alcohol over several hours, and made into paraffin blocks. The blocks were sliced into 4- μm thick sections and stained with hematoxylin and eosin. Four sites in the septal chondro-osseous junctions of each specimen were observed under high-power magnification ($\times 400$) under light microscopes by two uninformed observers who counted the eosinophils in each view.

Cytokines in the BAL Fluid and Total IgE in the Serum

To evaluate the allergic reaction with the BAL fluid (the supernatant from each group), interferon γ (IFN- γ) (R&D Systems, Minneapolis, MN) for Th1 immune reaction and interleukin (IL)-13 (R&D Systems) for Th2 immune reaction were measured with ELISA. Total IgE (Pharmingen, San Diego, CA) in the serum was measured with the ELISA kit.

Statistical Analysis

All measured parameters were expressed as means $\pm$ SD. The statistical parameters observed among the study groups were analyzed with a Kruskal-Wallis analysis of variance by ranks test. A Mann-Whitney U test was performed for the parameters proven statistically significant using SPSS version 10 (SPSS Inc, Chicago, IL).

RESULTS

Comparison of Allergic Symptoms

The mean symptom score, which is the total number of sneezing and nasal rubbing motions during 15 minutes after the final allergen inoculation, was 9.8 ± 1.7 in the control group, 41.7 ± 3.5 in the allergic rhinitis group, and 12.1 ± 1.7 in the BCG treatment group. The symptom score was elevated in the allergic rhinitis group compared with the control group ($P < 0.001$) and decreased in the BCG treatment group compared with the allergic rhinitis group ($P < 0.001$). Other comparisons between the two groups failed to show significance.

Eosinophilic Infiltration of the Nasal Mucosa

In the allergic rhinitis group, the number of lymphocytes and eosinophils was higher in the lamina propria compared with the mucosal epithelium (Fig 2). The lamina propria eosinophilic infiltration decreased in the BCG group. At

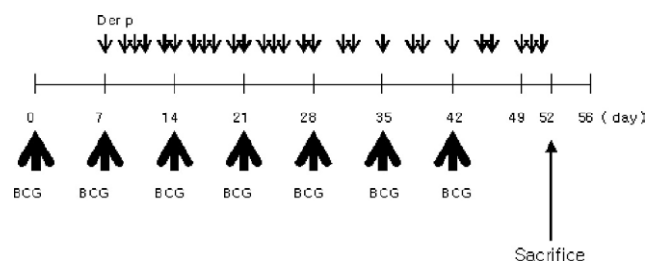


Figure 1 Schematic representation of the local sensitization and BCG treatment protocol.

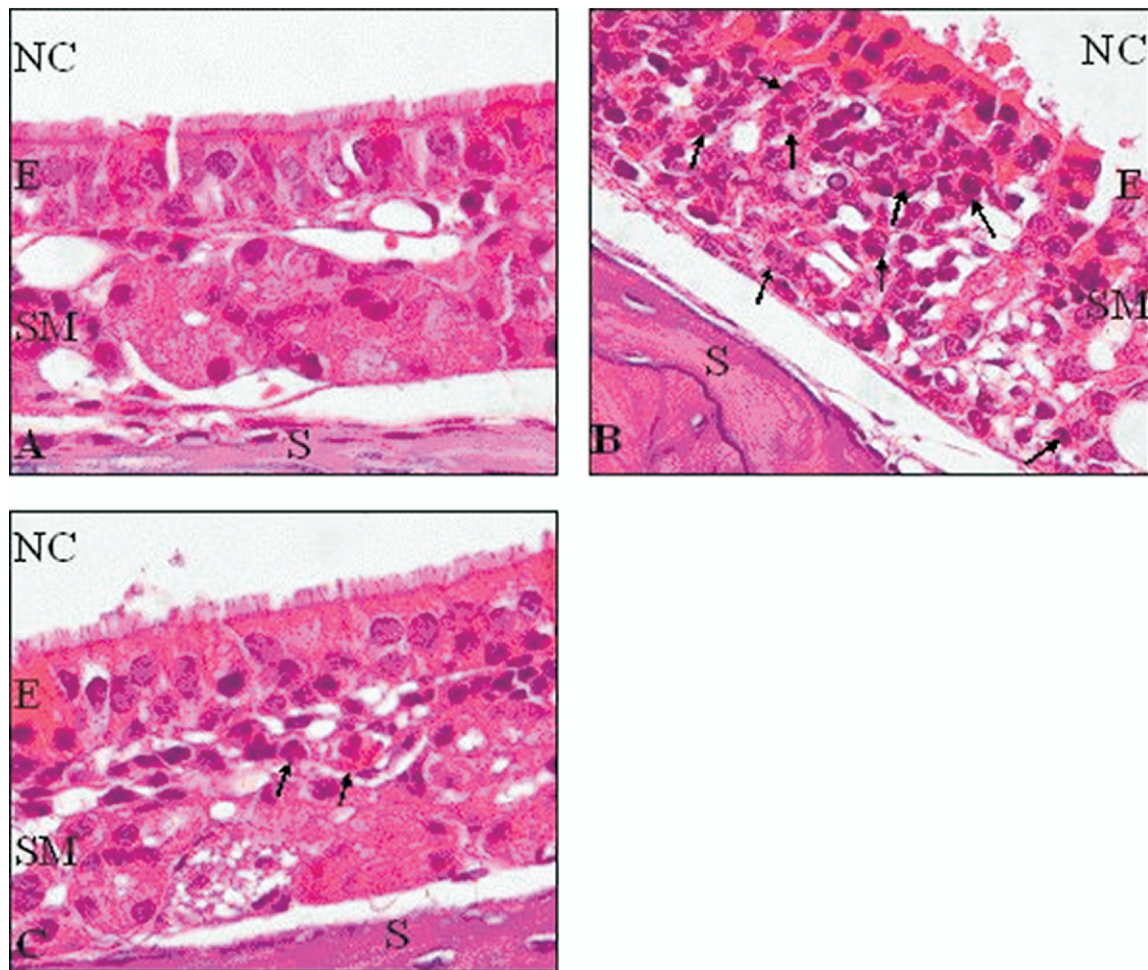


Figure 2 Infiltration of eosinophils (arrows) induced by *D pteronyssinus* in the nasal mucosa of BALB/c mice. (A) Control group, (B) allergic rhinitis group, and (C) BCG treatment group. NC, nasal cavity; E, epithelium; SM, submucosa; S, septum. (Hematoxylin and eosin stain; original magnification, $\times 1,000$).

$\times 400$ magnification, the eosinophil count per view of the nasal mucosa was 2.6 ± 0.3 in the control group, 23.8 ± 0.8 in the allergic rhinitis group, and 5.1 ± 0.5 in the BCG treatment group. The eosinophil count in the nasal mucosa increased significantly in the allergic rhinitis group compared with the control group ($P < 0.001$), whereas it decreased significantly in the BCG treatment group compared with the allergic rhinitis group ($P < 0.001$). Additional intergroup analyses failed to show statistical significance.

Serum Total IgE

Serum total IgE levels were elevated both in the allergic rhinitis group ($9.16 \pm 1.42 \mu\text{g/mL}$) ($P < 0.001$) and the BCG treatment group ($4.60 \pm 0.97 \mu\text{g/mL}$) ($P < 0.001$) compared with the control group ($0.31 \pm 0.01 \text{ g/mL}$). Although the BCG treatment group showed lower serum total IgE levels than the allergic rhinitis group, the difference was not statistically significant.

Inflammatory Cells within the BAL Fluid

In the control group, cell counts were as follows: total, $25.87 \pm 2.48 \times 10^4/\text{mL}$; macrophages, $25.78 \pm 2.48 \times$

$10^4/\text{mL}$; lymphocytes, $0.06 \pm 0.01 \times 10^4/\text{mL}$; and neutrophils, $0.01 \pm 0.01 \times 10^4/\text{mL}$. In the allergic rhinitis group, cell counts were as follows: total, $160.50 \pm 15.24 \times 10^4/\text{mL}$; macrophages, $70.66 \pm 8.05 \times 10^4/\text{mL}$; eosinophils, $64.93 \pm 17.10 \times 10^4/\text{mL}$; lymphocytes, $6.31 \pm 1.22 \times 10^4/\text{mL}$; and neutrophils, $18.58 \pm 3.23 \times 10^4/\text{mL}$. In the BCG treatment group, cell counts were as follows: total, $27.75 \pm 7.04 \times 10^4/\text{mL}$; macrophages, $10.86 \pm 1.51 \times 10^4/\text{mL}$; eosinophils, $11.78 \pm 4.76 \times 10^4/\text{mL}$; lymphocytes, $0.70 \pm 0.22 \times 10^4/\text{mL}$; and neutrophils $4.41 \pm 1.39 \times 10^4/\text{mL}$. The number of total cells, macrophages, eosinophils, lymphocytes, and neutrophils was significantly higher in the allergic rhinitis group compared with the control group ($P < 0.01$) and significantly lower in the BCG treatment group compared with the allergic rhinitis group ($P < 0.01$) (Figs 3 and 4).

Cytokine Analysis of the BAL Fluid

IFN- γ levels in the BAL fluid significantly decreased in the allergic rhinitis group ($1.52 \pm 0.11 \text{ pg/mL}$) compared with the control group ($2.42 \pm 0.14 \text{ pg/mL}$) ($P < 0.001$) and significantly increased in the BCG treatment group ($3.25 \pm$

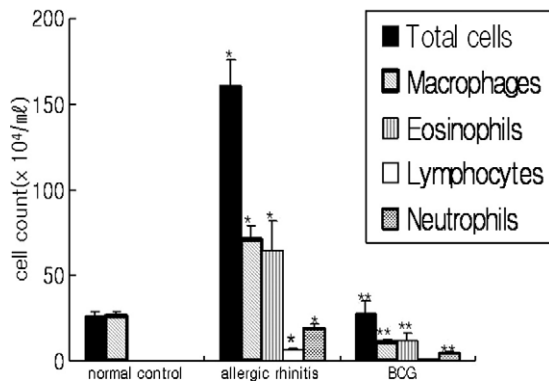


Figure 3 Inflammatory cells in BAL fluid of each group. Compared with the control group, the allergic rhinitis group showed a significant increase in the number of inflammatory cells, including eosinophils, lymphocytes, and neutrophils. Compared with the allergic rhinitis group, the BCG treatment group showed a significant decrease in the number of inflammatory cells, including eosinophils, lymphocytes, and neutrophils. * $P < 0.01$ versus the control group. ** $P < 0.01$ versus the allergic rhinitis group.

0.57 pg/mL) compared with the allergic rhinitis group ($P = 0.031$). IL-13 levels in the BAL fluid were significantly elevated in the allergic rhinitis group (36.75 ± 13.39 pg/

mL) ($P = 0.046$) and the BCG treatment group (17.21 ± 1.82 pg/mL) ($P < 0.001$) compared with the control group (6.80 ± 0.76 pg/mL). The BAL fluid in the BCG group showed a lower level of IL-13 than in the allergic rhinitis group, although the difference was not statistically significant (Fig 5).

DISCUSSION

Allergic rhinitis is closely related to allergic asthma, and 88% of patients with asthma have symptoms of rhinitis as well as positive skin tests.⁶ Animal models for both allergic diseases are produced in a similar manner. However, whereas a variety of models have been developed for allergic asthma, relatively few exist for allergic rhinitis.

The results of the present study showed that allergic symptoms, tissue eosinophilia, serum total IgE, and eosinophilia and IL-13 levels in the allergic rhinitis group were significantly higher compared with the control group. These findings suggest that we successfully produced an animal model of allergic rhinitis via local sensitization of mice with the house dust mite allergen. McCusker et al⁶ reported that allergic inflammation can be induced in both the upper and

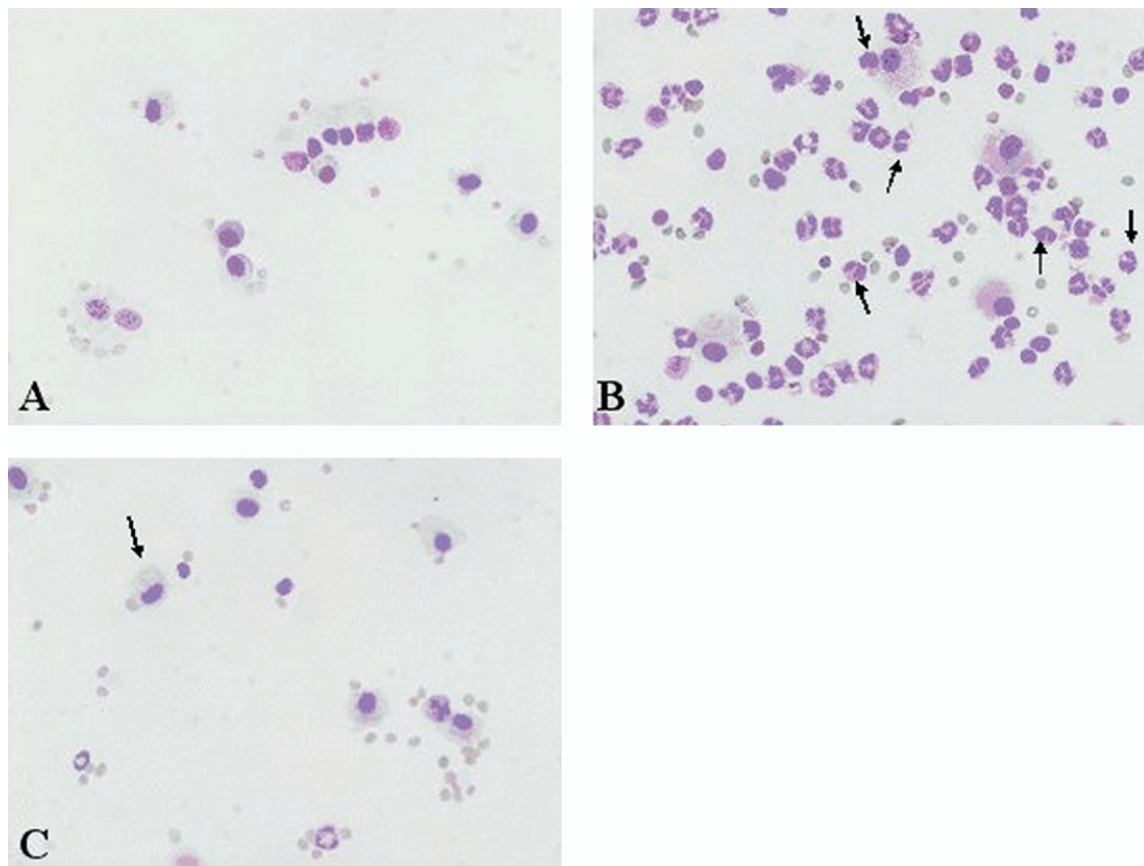


Figure 4 Photomicrographs of Diff-Quik-stained bronchoalveolar lavage cells in each study group (original magnification, $\times 400$). (A) Control group, (B) allergic rhinitis group, and (C) BCG treatment group. Compared with the control group, the allergic rhinitis group showed an increased number of inflammatory cells, including eosinophils (arrows), lymphocytes, and neutrophils. In the BCG treatment group, the number of eosinophils decreased compared with the allergic rhinitis group.

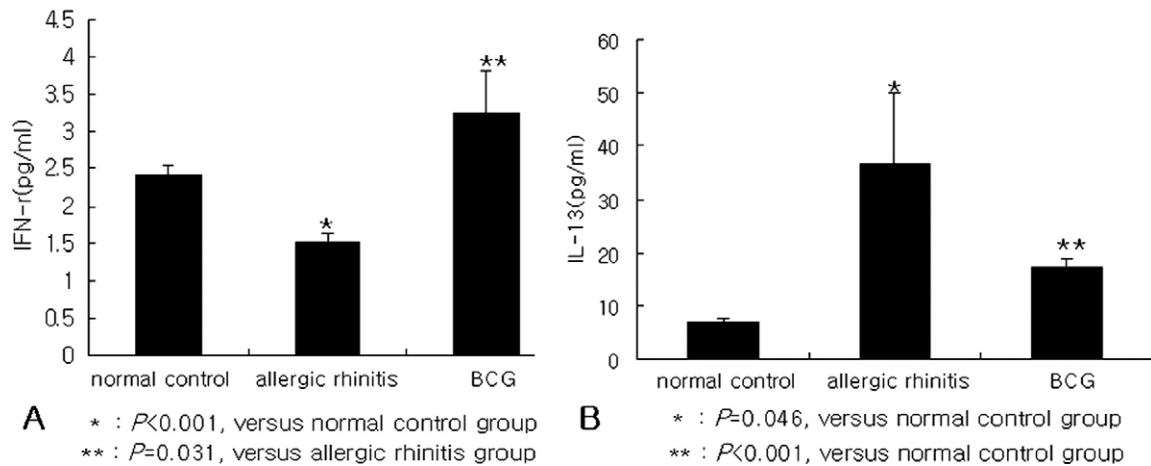


Figure 5 Cytokine levels in the BAL fluid of each study group. (A) IFN- γ and (B) IL-13.

lower respiratory tract via local sensitization intranasally with ovalbumin and that most of the allergen inoculated in this way are precipitated in the nasal mucosa. Ovalbumin has long been used to produce animal models of allergy with mice, white rats, and guinea pigs; however, this approach offers limited clinical utility because ovalbumin is an uncommon allergen. In the present study, we used mice to develop the animal model because the immunological information is relatively well known. House dust mites, including *D pteronyssinus* and *Dermatophagoides farinae*, are allergens commonly used in the development of allergic models. Cheng et al⁷ produced an allergic rhinitis mouse model with *D pteronyssinus*, and Yun et al⁵ compared systemic sensitization with local intranasal sensitization using *Dermatophagoides farinae* and concluded that local sensitization after systemic sensitization is more effective than local sensitization alone. However, the former study differed from the present study in that the allergen was mixed with an adjuvant and the allergen inoculation was performed only once a week for six weeks. Animal allergy models differ among laboratories in terms of the type, amount, and concentration of allergens used, as well as the method, frequency, and total duration of allergen inoculation.⁸ Johnson et al⁹ developed a model of allergic asthma via inoculation with the house dust mite and ovalbumin allergens intranasally five times each per week for seven weeks. Cytokine levels related to the Th2 response increased, but nine weeks after cessation of allergen exposure, the airway inflammatory response resolved. Our murine allergic rhinitis model included 27 inoculations over a period of seven weeks; however, further study is needed to evaluate the time required for the resolution of allergic inflammation.

Our results showed that allergy symptom scores, mucosal eosinophilia, serum total IgE, BAL fluid inflammatory cells, and IL-13 decreased in the BCG treatment group compared with the allergic rhinitis group, but that IFN- γ increased. These findings strongly suggest that allergic rhinitis is suppressed by BCG. Because most allergic diseases

develop from reinforcement of the Th2 response elicited by an allergen, recent studies have focused on strengthening the Th1 response to suppress the Th2 response as a novel treatment for allergic diseases.^{3,10} Recent reports suggest that viral or bacterial infection strengthens the Th1 response, which consequently will suppress the allergic reaction. The *Mycobacterium* species and BCG, the vaccine used to prevent tuberculosis, are known to elicit a strong Th1 response. Neonatal BCG vaccination may decrease the incidence of allergic diseases,¹¹ and Koh et al¹² suggested that because all newborns in Korea are vaccinated with BCG at four weeks of age, children with atopic genetic traits may avoid developing allergic rhinitis or allergic asthma.

After administration of BCG in an allergic asthma model, Yang et al³ reported that asthmatic symptoms were reduced via alterations in cytokines and cell adhesion molecule expression; however, no significant effect on IgE levels was observed. In the present study, serum total IgE levels in the BCG treatment group decreased compared with the allergic rhinitis group, although the difference was not statistically significant.

The antiallergic mechanism of BCG has been described in the literature.^{10,13} The CpG oligodeoxynucleotides of BCG stimulate the antigen-presenting cells, causing them to secrete cytokines such as IL-12 and IFN- γ ; these in turn activate natural killer cells and induce a strong Th1 immune reaction, thereby suppressing allergic inflammation. CpG oligodeoxynucleotides may also suppress IgE production by directly acting on B cells, thereby suppressing the Th2 reaction.

Previous studies have evaluated the effect of various BCG concentrations as well as the timing and route of administration in murine models. Erb et al¹⁴ reported that the antiallergic effect of BCG increased in proportion to the concentration of BCG administered, that BCG was most effective when administered before antigen sensitization, and that local intranasal inoculation was more effective than transdermal injection. Koh et al¹² showed that a sufficient

concentration of BCG may produce an antiallergic effect even during the time of sensitization.

CONCLUSIONS

After repeated intranasal sensitization with the house dust mite allergen, we were able to produce an animal model of allergic rhinitis. With this model, we successfully showed a reduction in allergic inflammation after administration of BCG. Our results suggest that BCG can play a role in the prevention and treatment of allergic rhinitis. Additional animal studies are warranted to evaluate the persistence of the allergic response after cessation of allergen exposure to further elucidate immune tolerance, airway remodeling, optimal BCG administration, and potential adverse reactions.

REFERENCES

1. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *J Allergy Clin Immunol* 2001;108:S2–8.
2. Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;1:1572–3.
3. Yang X, Fan Y, Wang S, et al. Mycobacterial infection inhibits established allergic inflammatory responses via alteration of cytokine production and vascular cell adhesion molecule-1 expression. *Immunology* 2002;105:336–43.
4. Hopfenspirger MT, Agrawal DK. Airway hyperresponsiveness, late allergic response and eosinophilia are reversed with mycobacterial antigens in ovalbumin-prensensitized mice. *J Immunol* 2002;168:2516–22.
5. Yun JB, Lee CH, Kwon TY, et al. House dust mite allergic rhinitis model in C57BL/6 mice. *Korean J Otolaryngol* 2000;43:276–81.
6. McCusker C, Chicoine M, Hamid Q, et al. Site-specific sensitization in a murine model of allergic rhinitis: role of the upper airway in lower airways disease. *J Allergy Clin Immunol* 2002;110:891–8.
7. Cheng CK, Lee KM, Krug MS, et al. House dust mite-induced sensitivity in mice. *J Allergy Clin Immunol* 1998;101:51–9.
8. Leong KP, Huston DP. Understanding the pathogenesis of allergic asthma using mouse models. *Ann Allergy Asthma Immunol* 2001;87:96–110.
9. Johnson JR, Wiley RE, Fattouh R, et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 2004;169:378–85.
10. Major T, Wohlleben G, Reibetanz B, et al. Application of heat killed *Mycobacterium bovis*-BCG into the lung inhibits development of allergen-induced Th2 responses. *Vaccine* 2002;20:1532–40.
11. Cunha SS, Cruz AA, Dourado I, et al. Lower prevalence of reported asthma in adolescents with symptoms of rhinitis that received neonatal BCG. *Allergy* 2004;59:857–62.
12. Koh YI, Choi IS, Park SC, et al. BCG infection during pre-sensitization or even post-sensitization inhibits airway sensitivity in an animal model of allergic asthma. *J Korean Med Sci* 2000;15:265–72.
13. Hussain I, Jain VV, Kitagaki K, et al. Modulation of murine allergic rhinosinusitis by CpG oligodeoxynucleotides. *Laryngoscope* 2002;112:1819–26.
14. Erb KJ, Holloway JW, Sobeck A, et al. Infection of mice with BCG suppresses allergen-induced airway eosinophilia. *J Exp Med* 1998;187:561–9.