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Effects of BCG infection on Schultz-Dale reaction, allergen-specific IgE levels, and Th2 immune response in sensitized rats

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Background : *BCG, a potent inducer of Th1 immune response, has been suggested to suppress Th2 response which is known to mediate IgE-mediated allergic disorders, in particular allergic asthma. Schultz-Dale reaction is known to be a model of IgE-mediated hypersensitivity. This study was done to investigate whether BCG infection suppresses the Schultz-Dale reaction by inhibiting Th2 response and allergen-specific IgE production.*

Methods : *Twenty-four Sprague-Dawley rats were sensitized and provoked with ovalbumin (OVA). A pretreatment of 6×10^4 colony forming units of BCG or saline was done 7 days before sensitization. The Schultz-Dale reaction was represented as tracheal smooth muscle contractions to 50 μg/mL OVA challenge in vitro. Serum OVA-specific IgE levels and IFN-γ and IL-4 concentrations in bronchoalveolar lavage fluid (BALF) were measured.*

Results : *The Schultz-Dale reaction and serum OVA-specific IgE levels were significantly decreased in BCG infected and OVA sensitized rats compared with only sensitized rats ($p<0.01$ and $p<0.05$, respectively). As compared with only sensitized rats, IL-4 concentration and a ratio of IFN-γ : IL-4 in BCG infected and OVA sensitized rats were significantly decreased ($p<0.001$) and increased ($p<0.05$), respectively. The Schultz-Dale reaction was correlated with OVA-specific IgE levels ($r=0.50$, $p<0.05$), IL-4 concentration ($r=0.69$, $p<0.001$), and ratio of IFN- : IL-4 ($r=-0.44$, $p<0.05$). OVA-specific IgE levels were correlated with IL-4 concentration ($r=0.61$, $p<0.01$) and ratio of IFN-γ : IL-4 ($r=-0.48$, $p<0.05$).*

Conclusion : *These findings suggest that BCG infection prior to allergen sensitization may inhibit Schultz-Dale reaction developed in the sensitized rat tracheal smooth muscle via the suppressive effects of Th2 immune response and allergen-specific IgE production.*

Key Words : *BCG vaccine; Asthma; Schultz-Dale reaction; Cytokines.*

INTRODUCTION

Immunoglobulin-E (IgE)-mediated allergic disorders, in particular allergic asthma, are mediated primarily by Th2 lymphocytes[1]. Th1 and Th2 cells reciprocally regulate each other through the cytokines they secrete. For example, interferon-γ (IFN-γ) and interleukin-12 (IL-12) inhibit Th2 cells and IL-4 and IL-10 down-regulate the activity of Th1 cells[1, 2]. In particular, IL-4 serves as a proliferation factor for Th2 cells and provides B cells help for allergen-specific IgE antibody production[3, 4]. Therefore, Th1 immune response may suppress allergen-specific IgE production via inhibition of IL-4 production.

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Considerable interest has been focused on the Schultz-Dale model of IgE-mediated hypersensitivity reaction which occurs in a diversity of animal tissues[5]. It is a local anaphylactic response initiated when mast cell-bound IgE antibodies are cross-linked by sensitizing antigen, leading to release of various chemical mediators[5]. It is possible that Th1 immune response inhibits the Schultz-Dale reaction by reducing allergen-specific IgE levels.

Mycobacterium species, including bacille Calmette-Guerin (BCG) used as a vaccine to prevent human tuberculosis, are known to be potent inducers of Th1 response[6,7]. Recent experimental studies have demonstrated that mycobacterial infections inhibit Th2 immune responses and allergen-specific IgE levels in allergen-sensitized mice[8-11]. We also have shown that BCG infection inhibits airway responsiveness and airway eosinophilia, which are characteristic of asthma[12], and Th2 immune responses[13] in our allergic asthma rats.

We hypothesized that BCG infection prior to allergen sensitization may inhibit the Schultz-Dale reaction to the allergen through suppression of Th2 immune response and the allergen-specific IgE production. Tracheal smooth muscle contractions to ovalbumin (OVA) challenge *in vitro* (i.e., Schultz-Dale reaction), serum OVA-specific IgE levels, and IFN-γ and IL-4 concentrations in bronchoalveolar lavage fluid were measured in BCG or saline infected and OVA sensitized rats.

MATERIALS AND METHODS

Experimental rats

Twenty-four male Sprague-Dawley rats 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 374.8±4.0 g (mean±SEM) body weight. They were sensitized with saline or OVA after saline or BCG infection, provoked with 5% OVA aerosols and divided into five different groups (Table 1). All procedures were reviewed and approved by the Committee on Animal Research at Chonnam National University Medical School and Chonnam University Hospital.

BCG infection

The rats were infected intraperitonealy with 6×10^4 colony forming units (CFUs) of live attenuated BCG (PASTEUR MERIEUX, France), or saline 7 days before OVA sensitization. The dose of BCG has been shown to suppress airway smooth muscle sensitivity of OVA sensitized rats in a previous study[12].

OVA sensitization

The rats were sensitized with a subcutaneous injection of 10 or 100 μg OVA (Grade III, Sigma), or saline together with 200 mg $Al(OH)_3$ and 1 mL killed Pertussis vaccine (1×10^{10}/mL, Korea Green Cross Corporation).

OVA provocation

Two weeks after OVA sensitization, all rats were provoked with 5% OVA 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.

Cytokine assays in bronchoalveolar lavage (BAL) fluids

The rats were euthanized by an intraperitoneal injection of 5 mg/kg thiopental sodium and exsanguinated through the inferior vena cava 24 hours after the provocation with OVA aerosols. The trachea was canulated with PE-20 polyethylene tube and BAL was

Table 1. Characteristics of each group

Group	N (24)	BCG (CFUs)	OVA sensitization	5 %-OVA provocation
Non-sensitized control	5	-	-	+
OVA sensitized control-Ⅰ	5	-	10 μg	+
OVA sensitized control-Ⅱ	5	-	100 μg	+
BCG infected and OVA sensitized -Ⅰ	5	+	10 μg	+
BCG infected and OVA sensitized -Ⅱ	4	+	100 μg	+

BCG, bacillus Calmette-Guerin; CFUs, colony forming units; N, the number of rats; OVA, ovalbumin

performed by 5 lavages with 4 mL physiologic saline (4℃). The recovered fluid was immediately centrifuged at 1500 rpm for 10 minutes at 4℃ (VS-6000, Vision, Korea). Cell-free BAL fluids were stored at -20℃ until analysis of cytokines. The concentrations of IL-4 and IFN-γ were determined by using commercially available ELISA kits (Endogen, MA, USA). The standard curves were generated by the standards of known IL-4 or IFN- content provided. Sensitivities were 2 pg/mL for IL-4 and INF-γ, respectively.

Tracheal smooth muscle responsiveness to OVA challenge *in vitro*

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 hours with washing at least every 20 minutes 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 50 μg/mL OVA was measured, which developed 2-3 min after OVA challenge *in vitro*. The segments were air-dried and weighed after experiment. The responses were expressed as a gram force/gram tissue (g/g).

Determination of OVA-specific IgE antibody levels

Blood was sampled from the inferior vena cava, clotted at room temperature and centrifuged at 1500 rpm for 10 min at 4℃ (VS-6000, Vision, Korea). Serum samples were stored at -20℃ until analysis. OVA-specific IgE levels were determined by coating microtiter plates with 100 μL of OVA (10 μg/mL). Rat sera were incubated in the antigen-coated wells at a final dilution of 1:100, and bound IgE was detected with a HRP-mouse anti-rat IgE (Zymed, California, USA). *O*-phenylenediamine substrate was added and developed, and the OD was measured at 450 nm.

Statistical analysis

All results were presented as means±SEM values. Student's *t*-test was used to determine the level of difference between the groups. Correlation analyses were performed by Pearson's correlation coefficient. A p value of less than 0.05 was considered significant.

RESULTS

Effect of BCG infection on Schultz-Dale reactions

No tracheal smooth muscle contractions to 50 μg/mL OVA were observed in non-sensitized rats. The muscle contractions in 10 or 100 μg OVA-sensitized rats (45.0± 9.1 g/g, n=10) were significantly increased as compared with the non-sensitized rats ($p<0.01$). The muscle contractions in BCG infected and OVA sensitized rats (4.1±2.7 g/g, n=9, $p<0.01$) were significantly decreased as compared with the sensitized rats. The muscle contraction in BCG infected and OVA sensitized rats did not differ from that in the non-sensitized rats ($p>0.05$) (Figure 1). There was no significant difference in the muscle contraction between 10 μg OVA sensitized rats (39.9± 15.3 g/g) and 100 μg OVA sensitized rats (50.1± 11.3 g/g, $p>0.05$). Also, BCG infected and 10 μg OVA sensitized rats did not significantly differ in the muscle contraction from BCG infected and 100 μg OVA sensitized rats.

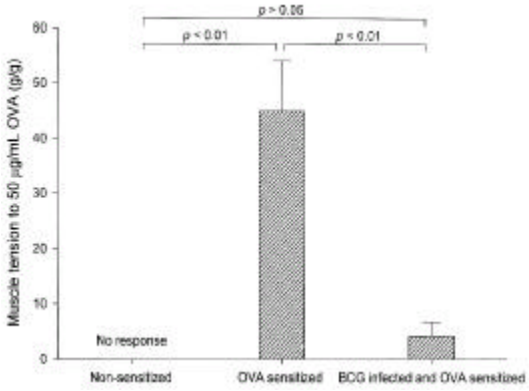

Figure 1. Tracheal smooth muscle contraction to 50 μg/mL ovalbumin (OVA) challenge *in vitro* in non-sensitized (n=5), OVA sensitized (n=10), and BCG infected and OVA sensitized rats (n=9).

Table 2. Concentrations of IFN-γ and IL-4 in bronchoalveolar lavage fluids

	Non-sensitized (n=5)	10 or 100 μg OVA sensitized (n = 10)	BCG infected and 10 or 100 μg OVA sensitized (n=9)
IFN-γ (pg/mL)	7.3±0.7	6.7±0.6	3.9±0.2†¶
IL-4 (pg/mL)	7.9±0.5	12.9±0.6*	5.1±0.8‡§

BCG, bacillus Calmette-Guerin; OVA, ovalbumin
*p<0.01 vs. non-sensitized rats. †p<0.01 and ‡p<0.001 vs. sensitized rats. ¶p<0.01 and §p<0.05 vs. non-sensitized rats.

Effect of BCG infection on Th2 immune response

Rats sensitized with 10 or 100 μg OVA showed a significant increase of IL-4 in BAL fluids (p<0.01) but no significant change of IFN-γ as compared with non-sensitized rats. The concentrations of IFN-γ and IL-4 were significantly decreased in BCG infected and OVA sensitized rats compared with the sensitized (p<0.01 and p<0.001, respectively) or non-sensitized rats (p<0.01 and p<0.05, respectively) (Table 2). Largely as a result of the increased or decreased IL-4 production, ratios of IFN-γ : IL-4 were significantly decreased in sensitized rats compared with non-sensitized rats (p<0.01) and significantly increased in BCG infected and OVA sensitized rats compared with the sensitized rats (p<0.05). The ratio did not significantly differ between non-sensitized rats and BCG infected and OVA sensitized rats (Figure 2). The 100 μg OVA sensitized rats showed non-significant decrease of IFN-γ levels (5.7±0.7 pg/mL vs. 7.7±0.6 pg/mL, p=0.07), significant increase of IL-4 levels (14.1±0.8 pg/mL vs. 11.6±0.5 pg/mL, p<0.05), and significant decrease of IFN-γ : IL-4 ratios (0.41±0.05 vs. 0.68±0.08, p<0.05) as compared with the 10 μg OVA sensitized rats. However, there were no differences in IFN-γ and IL-4 levels and IFN-γ : IL-4 ratios between BCG-infected & 10 μg OVA-sensitized rats and BCG-infected & 100 μg OVA-sensitized rats.

Effect of BCG infection on OVA-specific serum IgE levels

Serum OVA-specific IgE levels were significantly increased in 10 or 100 μg OVA sensitized rats compared with non-sensitized rats (p<0.01) and significantly decreased in BCG infected and OVA sensitized rats compared with the sensitized rats (p<0.05). The BCG infected and OVA sensitized rats did not differ in OVA-specific IgE levels from non-sensitized rats (p>0.05) (Figure 3). There was no difference in OVA-specific IgE levels between 10 μg OVA sensitized rats (0.26±0.02 OD) and 10 μg OVA sensitized rats (0.28±0.01 OD, p>0.05) or between BCG-infected & 10 μg OVA-sensitized rats (0.23±0.02 OD) and BCG-infected & 100 μg OVA-sensitized rats (0.20±0.01, p>0.05).

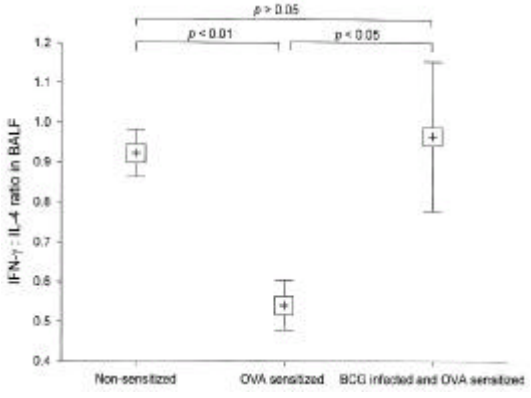

Figure 2. A ratio of IFN-γ : IL-4 in bronchoalveolar lavage fluids (BALF) in non-sensitized (n=5), ovalbumin (OVA) sensitized (n=10), and BCG infected and OVA sensitized rats (n=9).

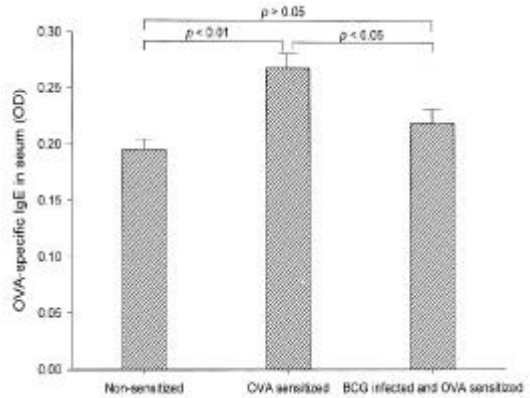

Figure 3. Serum ovalbumin (OVA)-specific IgE level in non-sensitized (n=5), ovalbumin (OVA) sensitized (n=10), and BCG infected and OVA sensitized rats (n=9).

Relationships among Schultz-Dale reaction, Th2 immune response and OVA-specific IgE level

The muscle contractions to 50 μg/mL OVA were correlated with serum OVA-specific IgE level ($r=0.50$, $p<0.05$, Figure 4A), IL-4 concentration in BAL fluid ($r=0.69$, $p<0.001$, Figure 4B), and IFN-γ : IL-4 ratio ($r=-0.44$, $p<0.05$, Figure 4C). The OVA-specific IgE level was correlated with IL-4 concentration ($r=0.61$, $p<0.01$, Figure 4D) and IFN-γ : IL-4 ratio ($r=-0.48$, $p<0.05$).

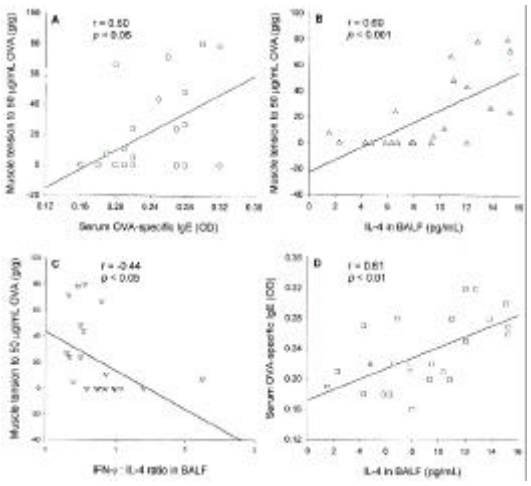

Figure 4. Relationships between tracheal smooth muscle contraction to 50 μg/mL ovalbumin (OVA) challenge *In vitro* and (A) serum OVA-specific IgE level, (B) IL-4 concentration in bronchoalveolar lavage (BALF) or (C) ratio of IFN-γ : IL-4 in BALF and (D) relationship between serum OVA-specific IgE level and IL-4 in BALF in all experimental rats.

DISCUSSION

This study demonstrated that BCG infection prior to allergen sensitization suppressed the Schultz-Dale reaction developed in the sensitized rat tracheal smooth muscle. The suppressive effect of BCG infection on the reaction was accompanied by reductions of serum allergen-specific IgE level and Th2 immune response. Furthermore, there were positive correlations among the Schultz-Dale reaction, allergen-specific IgE levels and IL-4 concentrations. These findings suggest that BCG infection may suppress the Schultz-Dale reaction by inhibiting Th2 immune responses and reducing allergen-specific IgE levels.

The Schultz-Dale reaction has been known to be the model of IgE-mediated allergic response which occurs in a diversity of animal tissues[5] and is a local anaphylactic response initiated when mast cell-bound IgE antibodies are cross-linked by sensitizing antigen, leading to release of various chemical mediators[5]. Mitchell et al.[14] have reported that ragweed-pollen-sensitized canine tracheal smooth muscle develops active tension in response to specific antigen challenge. Many studies have indicated that histamine is one of the major mediators released from mast cells during the Schultz-Dale reaction[14-18]. Therefore, the presence or degree of the Schultz-Dale reaction is likely to be related to the allergen-specific IgE levels, which was supported by our finding that the degree of Schultz-Dale reaction correlated positively with serum allergen-specific IgE antibody levels. Accordingly, it is possible that the Schultz-Dale reaction is suppressed by reducing the mast cell-bound allergen-specific IgE antibodies. Our study showed that BCG infection significantly decreased the allergen-specific IgE levels in allergen sensitized rats. These findings suggest that the inhibition of the Schultz-Dale reaction by BCG infection may be mediated through the suppressive effect of BCG infection on the allergen-specific IgE levels.

How can BCG infection reduce the allergen-specific IgE levels? *Mycobacterium* species, including BCG, are known to be potent inducers of Th1 response[6,7]. Because Th1 and Th2 cells reciprocally regulate each other through the cytokines they secrete[1,2], BCG immunization is most likely to suppress Th2 immune response. The present study showed that ratio of IFN-γ : IL-4 was significantly increased in BCG infected and OVA sensitized rats compared with only OVA sensitized rats, being indicative of the suppressive effect of BCG infection on Th2 immune response. It has been known that IL-4, a Th2 cytokine, provides B cells help for allergen-specific IgE antibody production[3,4], which might explain the reason why serum OVA-specific IgE levels correlated with IL-4 concentration in the present study. Our study showed that IL-4 levels in BAL fluid were significantly reduced in BCG infected and OVA sensitized rats compared with only OVA sensitized rats, suggesting that the reduction of allergen-specific IgE levels by BCG infection was mediated via the suppressive effects of BCG infection on IL-4 production or Th2 immune response.

In the present study, although BCG infection increased IFN-γ : IL-4 ratio and decreased IL-4 levels in the sensitized rats, IFN-γ levels were rather decreased. It is not clear why the IFN-γ levels were not increased but decreased. However, it is tempting to speculate that the

balance between IL-4 and IFN-γ present at the site of T-cell activation determines whether Th1 or Th2 cell is generated, with relatively low IL-4 levels being susceptible to the inhibitory effect of IFN-γ on the Th2 development. It has been suggested that the ratio of Th1-Th2 cytokine production elicited by allergen exposure was critical in determining the type of immune response[19].

In addition, two doses (10 or 100 μg) of OVA sensitization were used in our experiment to study whether the suppressive effects of BCG infection could differ according to the doses of sensitization. Although 100 μg OVA sensitization produced greater Th2 immune response than 10 μg OVA sensitization, doses of 6×10^4 CFUs of BCG were sufficient to suppress the Schultz-Dale reaction, allergen-specific IgE production and Th2 immune response, regardless of the two different sensitized doses.

Taken together, we demonstrated that BCG infection prior to allergen sensitization may inhibit the Schultz-Dale reaction, a model of IgE-mediated response, developed in the sensitized rat tracheal smooth muscle, via the suppressive effect of Th2 immune response and allergen-specific IgE production. These findings suggest that BCG vaccine given at 4 weeks of life for preventing tuberculosis in Korea may be helpful in hindering the development of allergic disorders, in particular allergic asthma. However, according to epidemiological studies[20, 21], the suppressive effect of BCG vaccine on the development of atopic disorder in children is debatable[22]. 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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