

Subcutaneous Vaccination of *Mycobacterium bovis* Bacillus Calmette-Guérin Attenuates Allergic Inflammation in a Murine Model of Asthma

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ABSTRACT

Background: The increase in the incidence of allergic disorders especially in developed countries may be explained by the hygiene hypothesis. A novel therapeutic approach that causes a Th1-dominant response under conditions of Th2-skewed immunity has been investigated. *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) is a widely used anti-tuberculosis vaccine that strongly induces a Th1-predominant immune response. A potential role for BCG in the treatment of allergic diseases has been reported. In the present study, we investigated whether subcutaneous inoculation with a low dose (1×10^5) of BCG vaccine can attenuate allergic inflammation of the airway in a murine model of asthma.

Methods: Female BALB/c mice were vaccinated on day 0 with a subcutaneous injection of 1×10^5 CFU of BCG, and sensitized to ovalbumin (OVA, i.p., with alum) on day 7 and 14, respectively. After a 2-week interval, they were exposed to aerosolized OVA (1%). In another experiment, BCG-sensitized splenic CD4⁺ T cells were adoptively transferred before sensitization.

Results: We found that BCG-vaccinated mice had fewer eosinophils in BALF and less severe allergic inflammation. The expression of IL-4 as well as Eotaxin and TARC was down-regulated in the vaccinated mice, while that of both IL-12 and IFN- γ was up-regulated. Moreover, the transfer of splenic CD4⁺ T cells from vaccinated mice into naïve mice before OVA-sensitization prevented the development of allergic inflammation. These splenic CD4⁺ T cells produced much IFN- γ .

Conclusions: The subcutaneous administration of (low-dose) BCG vaccine suppressed allergic inflammation via Th1-skewed immunity and might have clinical applications in immunotherapy for asthma.

KEY WORDS

asthma, CD4⁺ T cell, chemokine, *Mycobacterium bovis*, Th1/Th2

INTRODUCTION

Epidemiological studies have proposed that the increase in the prevalence of atopic diseases correlates with a decrease in the incidence of infectious diseases such as tuberculosis.¹ This explanation has led to the 'hygiene hypothesis', which suggests that the increase in allergic diseases is caused by a cleaner environment and fewer childhood infections.²⁻⁴ A

number of reports support this hypothesis, demonstrating that allergic disorders, like bronchial asthma, have been increasing rapidly in developed countries and favor Th2-skewed rather than Th1-skewed immunity.⁵⁻¹⁰ It was believed that there is a reciprocal balance between Th1 cytokines including IFN- γ , TNF- α and IL-12, and Th2 counterparts such as IL-4, IL-5 and IL-13. Th2 cytokines induce immunoglobulin (Ig) E synthesis and eosinophilic inflammation, while Th1

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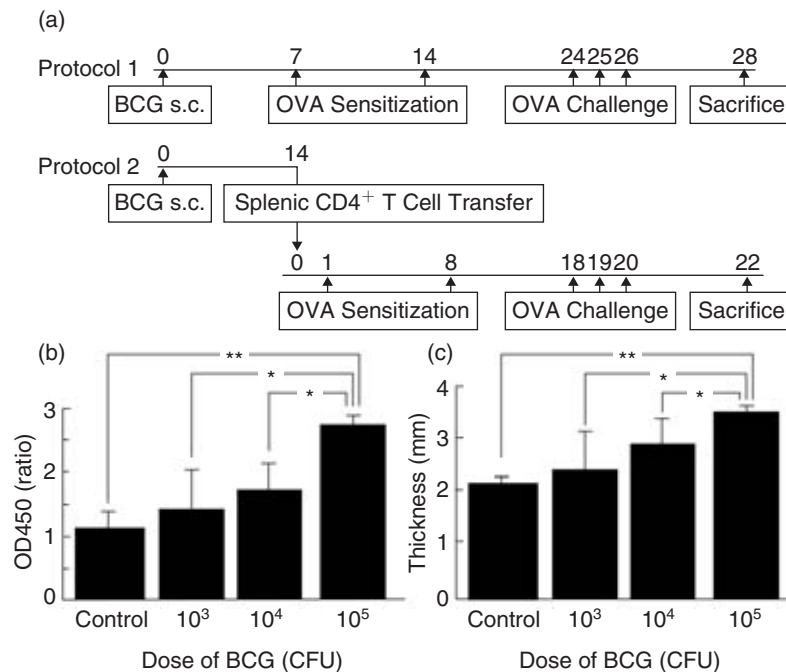


Fig. 1 Experimental design used to investigate the effect of BCG vaccine on OVA-induced bronchial asthma. **(a)** Schematic representation of the experimental protocol. Mice were sensitized and challenged as described in the Methods. Mice were treated with BCG or BCG-sensitized splenic CD4⁺ T cells before sensitization as indicated in the Methods. **(b)** Determination of BCG dose using the MTT assay. **(c)** Determination of BCG dose using DTH. Data are the means $\pm$ SEM from four mice in each group. * $p < 0.05$, ** $p < 0.01$ compared with 1×10^4 CFU of BCG-immunized mice, control mice, respectively.

cells, through the secretion of IL-12 and IFN- γ , play a role in anti-mycobacterial immune responses by activating macrophages and protecting against allergic diseases by dampening Th2 cytokine functions.¹¹⁻¹³ Chemokines, which regulate the extravasation and accumulation of leucocytes during immune and inflammatory responses, are involved in the pathogenesis of allergic disorders.^{14,15} For example, Eotaxin^{16,17} and TARC¹⁸ play a pivotal role in allergic inflammation of the airway. Some researchers reported that vaccinations, such as measles¹⁹, pertussis²⁰ and tuberculosis,^{21,22} can cause a shift from a Th2 to Th1 milieu, which may protect against the development of allergies. *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), a vaccine used to prevent tuberculosis, is also reported to be a potent inducer of Th1-skewed immunity.²³ Some reports indicated that BCG treatment suppresses allergen-induced inflammation of the airway and hyperresponsiveness in mice.²⁴⁻²⁷ However, in these previous studies, BCG (1×10^5 – 1×10^7 CFU) was administered via the intranasal, intravenous, or intraperitoneal route in mice.²⁵⁻²⁸ Normally, BCG is inoculated subcutaneously at 10^7 – 10^8 CFU/human. In this study, we investigated whether BCG subcutaneous (s.c.) vaccination with a lesser dose

(1×10^5 CFU) can inhibit allergic inflammation induced by exposure to an allergen, change cytokine production from a Th2 to Th1-dominant immune profile, and regulate chemokines such as Eotaxin and TARC. Possible roles of BCG vaccine in the development of bronchial asthma are discussed.

METHODS

ANIMALS

Female BALB/c mice, originally obtained from Shizuoka Laboratory Animal Center (Shizuoka, Japan), were maintained in our animal facility at Nara Medical University. Female 6-week-old mice were used for experiments. All experimentation was conducted under a protocol approved by our institutional review board.

MYCOBACTERIUM (*M.*) BOVIS BCG

M. bovis BCG (Pasteur strain) was grown in Middlebrook 7H9 broth (Difco, Detroit, MI, USA) supplemented with Middlebrook OADC enrichment (Life Technologies, Gaithersburg, MD, USA) for 2 weeks at 37°C. This strain was centrifuged at 8000 rpm for 30 minutes at 4°C, and the precipitate was washed with saline and stored frozen as 1 ml aliquots at –80°C.

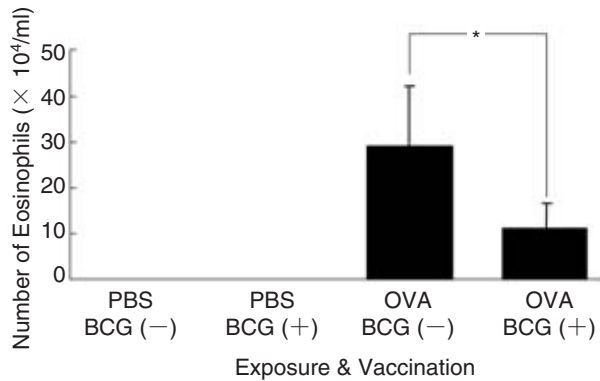


Fig. 2 Effect of vaccination on the number of eosinophils recovered in the BALF. BALF was collected 48 hours after the final challenge. Data are the means $\pm$ SEM from five mice in each group. The OVA-sensitized/challenged mice vaccinated with BCG had significantly fewer eosinophils in the BALF (* $p < 0.02$).

prior to use. As for the concentration of BCG, the number of colony forming units (CFU) per ml was determined by dispersing the bacteria through exposure to sonic oscillation and by plating suitable saline dilutions on Middlebrook 7H10 agar medium.

3-(4,5-DIMETHYL-2-THIAZOLYL)-2,5-DIPHENYLTETRAZOLIUM BROMIDE (MTT) ASSAY

The biological response of spleen lymphocytes was evaluated using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium Bromide (MTT) assay (Nacalai tesque Inc. Kyoto, Japan) according to the manufacturer's instructions. Lymphocytes were obtained by preparing single-cell suspensions from spleens of mice 2 weeks after the BCG immunization. Cell suspensions were passed through nylon mesh. RBC in the spleen were lysed with an ammonium chloride solution (ACK). These splenic lymphocytes (2×10^5) were cultured with heat-killed BCG antigens (5×10^3) or saline in 200 μ l of RPMI 1640 medium containing 10% FCS and antibiotics in a round-bottomed 96-well plate. All experiments were carried out in triplicate. These cultures were incubated at 37°C in a humidified 5% CO₂ atmosphere for 72 hours. The cell viability was >97% in dye exclusion tests using trypan blue. MTT solution (10 μ l) was added to each plate. After 90 minutes' incubation at 37°C, the absorbance at 450 nm was read on a microplate reader.

DELAYED-TYPE HYPERSENSITIVITY (DTH)

DTH was evaluated using the footpad method. Briefly, 10 μ g of PPD in 10 μ l of saline was injected subcutaneously into the right hind footpad 20 days after the subcutaneous immunization of 1×10^4 or 1×10^5 CFU of BCG in 200 μ l of saline. Control mice

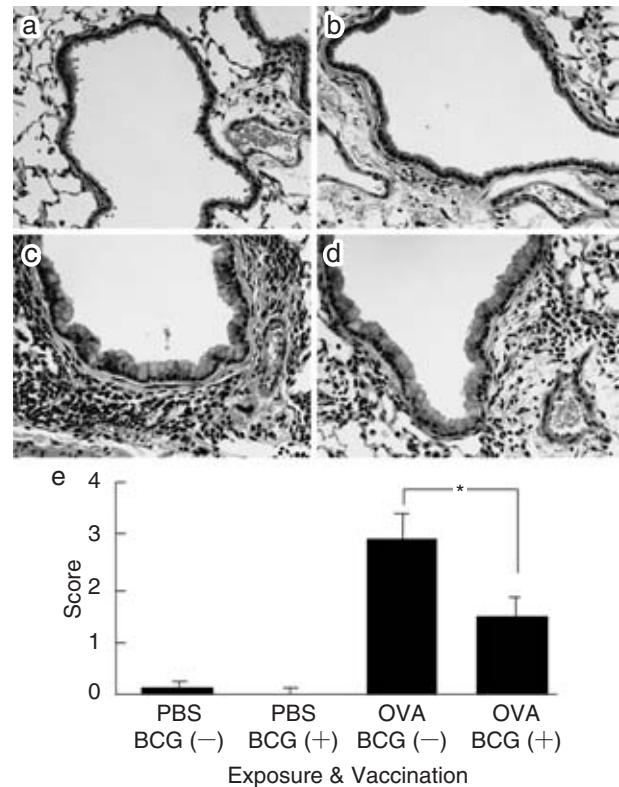


Fig. 3 Histopathologic analysis of lung tissue. (a) OVA-sensitized and PBS-exposed mice treated with vehicle (saline). (b) OVA-sensitized and PBS-exposed mice vaccinated with BCG. (c) OVA-sensitized and -exposed mice treated with vehicle. (d) OVA-sensitized and -exposed mice vaccinated with BCG. Histopathology showed accumulation of lymphocytes and eosinophils around airways and vessels and goblet cell hyperplasia in the lung tissue from OVA-sensitized and -exposed mice in the vehicle control. These pathologic changes were reduced in the lung tissue from BCG-vaccinated mice. (e) Quantitative analysis of histopathologic changes using a scoring system detailed in the methods. Data are the means $\pm$ SEM from five mice in each group. ($p < 0.05$).

were injected with saline. After 48 hours, footpad thickness was measured using a gauge micrometer (Mitutoyo, Tokyo, Japan) and the difference in thickness between the right and left hind footpads, the latter treated with saline alone as a control, was determined.

VACCINATION

Mice were vaccinated on day 0 with a s.c. injection of 1×10^5 CFU of BCG in 200 μ l of saline. Non-vaccinated control mice were injected only with saline (Fig. 1a).

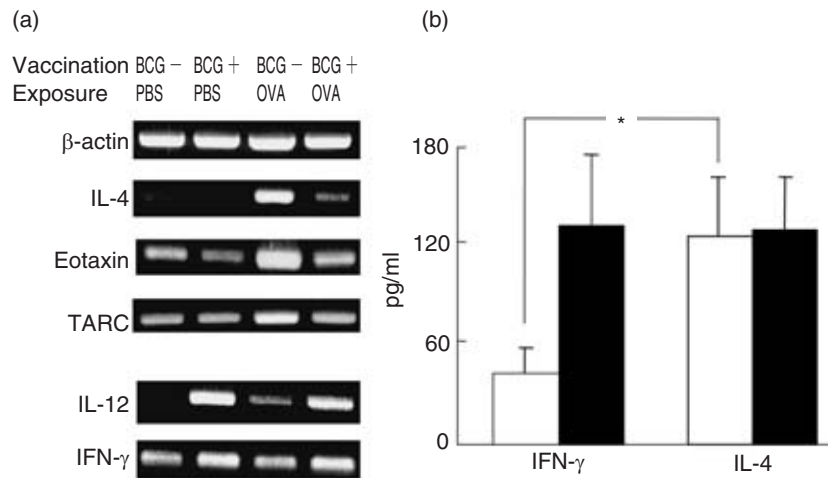


Fig. 4 The effect of BCG vaccine on production of cytokines and chemokines. (a) RT-PCR analysis of the lung from each mice described in the Methods. (b) Levels of IFN- γ and IL-4 measured by ELISA in the serum of OVA-sensitized and -exposed mice. Mice were treated with saline (□) or BCG (■). Data are the means $\pm$ SEM from five mice in each group (* $p < 0.01$).

SENSITIZATION AND EXPOSURE TO THE ALLERGEN

BALB/c mice were sensitized to ovalbumin (OVA) on day 7 (20 μ g with 2 mg of alum) and day 14 (10 μ g with 1 mg of alum). After a 2-week interval, mice were exposed to aerosolized OVA (1%, 10 min/day, for 3 consecutive days) or PBS (Fig. 1a).

BRONCHOALVEOLAR LAVAGE FLUID (BALF) COLLECTION

At 48 hours after the final challenge, the animals were euthanized with sodium pentobarbital (Nakalai Tesque Inc. Kyoto, Japan). The chest wall was opened and the animals were exsanguinated by cardiac puncture. Serum was collected and stored at -20°C prior to use. The trachea was cannulated after blood samples were collected. Bronchoalveolar lavage (BAL) was performed three times with 0.8 ml of sterile PBS instilled and harvested gently. Lavage fluid (the volume recovered was $\sim 85\%$ of that instilled) was collected and 1 ml was centrifuged at 2000 rpm (400 g) for 10 minutes. The cell pellet was resuspended in 0.25 ml of PBS. Total cell yield was quantified by hemocytometer. Differential cell counts were performed on cytocentrifuge slides prepared by centrifugation of samples at 800 rpm for 5 minutes (Autosmear CF12D, Sakura Seiki, Yao, Japan). These slides were fixed in 95% methanol and stained with Diff-Quick (Intl Reagent Corp, Kobe, Japan), modified Wright-Giemsa stain, and a total of 200 cells were counted for each sample by microscopy. Macrophages, lymphocytes, neutrophils, and eosinophils were enumerated.

HISTOLOGY

After lavage, some of the lungs were instilled with 10% buffered formalin, removed, and fixed in the same solution. After paraffin embedding, sections for microscopy were stained with H&E. An index of pathologic changes in coded H&E slides was established by scoring the inflammatory cell infiltrates around airways and vessels for severity (0, normal; 1, <3 cells thick; 2, 4–10 cells thick; 3, >10 cells thick) and overall extent (0, normal; 1, $<25\%$ of sample; 2, 25–50%; 3, 51–75%; 4, $>75\%$). The index was calculated by multiplying severity by extent, with a maximal possible score of 12.

ISOLATION OF RNA AND RT-PCR ASSAY

Whole lungs were removed for the isolation of RNA 48 hours after the last administration of OVA. Total RNA was isolated from whole lung using TRIZOL Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. It was then reversely transcribed into cDNA using a Superscript II preamplification kit (Invitrogen Life Technologies) and amplified with specific oligonucleotide primers as previously described.²⁹ Primers specific for β -actin (forward 5'-TGT GAT GGT GGG AAT GGG TCA G-3'; reverse 5'-TTT GAT GTC ACG CAC GAT TTC C-3'), IFN- γ (forward 5'-ACT GCC ACG GCA CAG TCA-3'; reverse 5'-GCG ACT CCT TTT CCG CTT-3'), IL-4 (forward 5'-GAA TGT ACC AGG AGC CAT ATC C-3'; reverse 5'-AAT CCA TTT GCA TGA TGC TCT T-3'), IL-12 (forward 5'-GAT GAC ATC ACC TGG ACC TCA-3'; reverse 5'-TCC TTC TTG TGG AGC AGC AG-3'), Eotaxin (forward 5'-AGA GCT CCA CAG CGC TTC TAT T-3'; reverse 5'-GGT

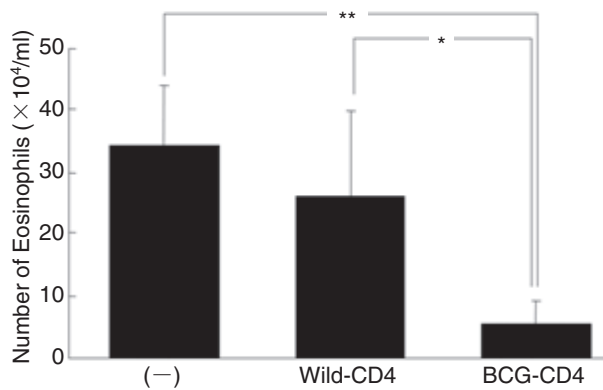


Fig. 5 Effect of BCG-sensitized CD4⁺ T cells on the number of eosinophils recovered in the BALF. At 14 days after the inoculation with BCG (1×10^5 CFU, s.c.) or saline, splenic CD4⁺ T cells (2×10^6) were adoptively transferred to naïve BALB/c mice via a tail vein. From the following day, sensitization and exposure to the allergen were performed. Adoptive transfer of BCG-sensitized CD4⁺ T cells significantly reduced the number of eosinophils in BALF in OVA-sensitized and -exposed mice. Data are the means $\pm$ SEM from five mice in each group (* $p < 0.02$, ** $p < 0.01$).

GCA TCT GTT GTT GGT GAT T-3'), and TARC (forward 5'- CAG GAA GTT GGT GAG CTG GTA TA-3'; reverse 5'- TTG TGT TCG CCT GTA GTG CAT A-3') were designed and synthesized at the Japan Bio Services (Saitama, Japan). PCR was performed for 35 cycles of 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 45 seconds, with the final extension done at 72°C for 10 minutes. The PCR products were examined by 2.5% agarose gel electrophoresis.

ADOPTIVE CD4⁺ T CELL TRANSFER EXPERIMENT

Fourteen days after the inoculation of BCG (1×10^5 CFU, s.c.) or saline, the entire spleen was removed. Following the lysis of RBC with ACK, splenic CD4⁺ T cells were isolated magnetically after incubation with anti-mouse CD4 (L3T4) mAb-coated magnetic beads (MACS; Miltenyi Biotec, Auburn, CA, USA). Cell purity was more than 95% throughout the experiments. These splenic CD4⁺ T cells (2×10^6) were adoptively transferred to naïve BALB/c mice via a tail vein. From the following day, sensitization and exposure to the allergen were performed. At 48 hours after the last exposure, mice were sacrificed for pathological analysis of the lungs (Fig. 1a).

CELL CULTURE

Isolated CD4⁺ T cells (2×10^5) were cultured in triplicate with heat-killed BCG (5×10^3) or saline in 200 μ l of RPMI 1640 medium containing 10% FCS and antibiotics in a round-bottomed 96-well plate as described. These cultures were incubated at 37°C in a humidi-

fied 5% CO₂ atmosphere. The culture supernatants for cytokine analysis were harvested 72 hours after culture and stored at -20°C prior to use.

CYTOKINE ELISA

The total amounts of IFN- γ and IL-4 in the serum and culture supernatant were measured by ELISA. ELISAs for IFN- γ and IL-4 were conducted according to the manufacturer's recommendations (Pierce Endogen, Rockford, IL). Standard curves were obtained using the standard samples provided. The sensitivity for each assay was 5 pg/ml.

STATISTICAL ANALYSIS

Statistical analysis was performed using Student's *t*-test. The 95% confidence limit was taken as significant. Results are expressed as means $\pm$ S.E.M. ($n = 4-5$)

RESULTS

DETERMINATION OF BCG DOSE

To determine the appropriate dosage of BCG for immunization, we used the MTT assay and footpad method. In the MTT assay, there was a significant difference in proliferative response to 1×10^5 CFU and 1×10^4 CFU or 1×10^3 CFU of BCG (Fig. 1b). Moreover, the mice that received 1×10^5 CFU of BCG showed a significant difference in footpad thickness compared with the control mice (Fig. 1c). Accordingly, we selected a dosage of 1×10^5 CFU for the vaccination.

CELLULAR PROFILES IN THE BALF

To quantitatively evaluate the cellular responses to the vaccination, we examined the cellular profiles in BALF. OVA-sensitized and exposed mice had increased eosinophil numbers in BALF compared with control mice sensitized to OVA and exposed to PBS. The vaccination significantly decreased the number of eosinophils in BALF ($29.4 \pm 12.5 \times 10^4$ vs. $10.9 \pm 5.4 \times 10^4$ /ml, $p < 0.02$) (Fig. 2).

HISTOPATHOLOGY OF LUNG TISSUE

To examine responses, a histological assessment of lung tissue from mice sacrificed at 48 hours after the last challenge was conducted. The mice showed robust pathologic changes due to allergic inflammation (eosinophil and mononuclear cell infiltration around the airway and vessels and goblet cell hyperplasia) (Fig. 3a). Histological evaluation of the lungs revealed a dampening of the pulmonary inflammation in the vaccinated mice (Fig. 3a). The OVA-sensitized/challenged mice that received the BCG vaccine showed significantly less cellular infiltration and goblet cell hyperplasia (2.93 ± 0.49 vs. 1.38 ± 0.27 , $p < 0.05$) (Fig. 3b).

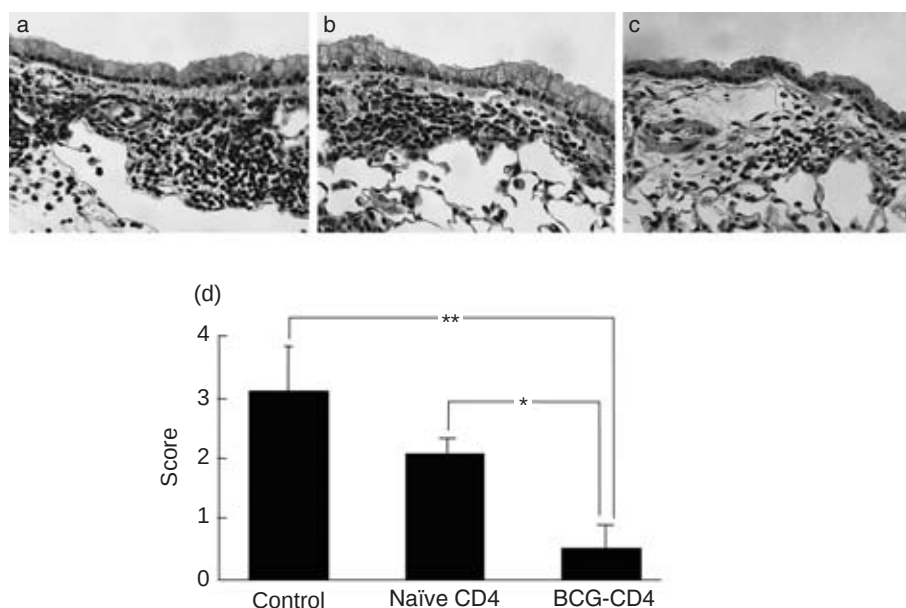


Fig. 6 Histopathologic analysis of lung tissue. PBS only (a), splenic CD4⁺ T cells from non-immunized mice (b), or splenic CD4⁺ T cells from vaccinated mice (c) were adoptively transferred to naïve BALB/c mice via a tail vein. They were sensitized and exposed to OVA as described in the Methods. The transfer of BCG-sensitized CD4⁺ T cells caused less extensive pathologic change in OVA-sensitized and -exposed mice (d) Histopathologic scoring is detailed in the Methods. Data are the means $\pm$ SEM from five mice in each group (* $p < 0.05$, ** $p < 0.01$).

EFFECT OF VACCINATION ON CYTOKINE AND CHEMOKINE PRODUCTION

To elucidate the mechanism by which allergic inflammation is inhibited by the vaccination, a RT-PCR-based analysis of whole lungs was performed. IL-4, Eotaxin and TARC gene expression levels were significantly lower in vaccinated/OVA-challenged mice than in non-vaccinated/OVA-challenged mice, while IL-12 and IFN- γ gene expression was upregulated after the vaccination regardless of exposure to OVA (Fig. 4a). Mice that received the BCG vaccine also showed higher levels of IFN- γ in serum (37.01 ± 16.25 vs. 126.0 ± 45.05 pg/ml, $p < 0.01$). On the other hand, there was no significant difference in IL-4 production (Fig. 4b). These results suggest that BCG vaccination stimulates the shift to a Th1 environment.

EFFECTS OF CD4⁺ T CELLS

To investigate whether helper CD4⁺ T cells, especially Th-1 cells, helped to attenuate allergic inflammation of the airway, splenic CD4⁺ T cells from the vaccinated mice were transferred into naïve mice before OVA-sensitization. Control mice received naïve CD4⁺ T cells or PBS only. The transfer of BCG-sensitized CD4⁺ T cells significantly reduced eosinophil numbers in BAL fluid in OVA-sensitized and -exposed mice (PBS, naïve CD4⁺ T cells, and BCG-sensitized CD4⁺ T cells; $33.7 \pm 9.63 \times 10^4$, $25.7 \pm 14.3 \times$

10^4 , and $5.13 \pm 3.65 \times 10^4$ /ml, respectively) ($p < 0.01$ vs. PBS, and $p < 0.02$ vs. naïve CD4⁺ T cells) (Fig. 5), and diminished the pathogenic changes of allergic inflammation (Fig. 6a). The histological score also decreased significantly on the transfer of BCG-sensitized CD4⁺ T cells. The score was 3.10 ± 0.69 ($p < 0.01$), 2.02 ± 0.24 ($p < 0.05$), and 0.54 ± 0.32 , respectively (Fig. 6b). IFN- γ gene expression was significantly higher, while IL-4 gene expression was lower with BCG-sensitized CD4⁺ T cells than non-sensitized CD4⁺ T cells, (Fig. 7a). Moreover, a much larger amount of IFN- γ was produced on the transfer of BCG-sensitized CD4⁺ T cells (92.4 ± 14.9 vs. 9556 ± 1819 pg/ml, $p < 0.01$) (Fig. 7b). IL-4 was undetectable.

DISCUSSION

The present study demonstrated that an inoculation of BCG vaccine was effective in reducing allergic inflammation of the airway in an OVA-sensitized murine model of asthma. Previous studies support the hypothesis that BCG immunization can promote Th1 immunity,³⁰ which has the potential to inhibit allergic disorders by attenuating Th2 immunity.³¹ However, the mechanism by which mycobacteria, such as BCG and *M. vaccae*, inhibit the inflammatory response has not been fully elucidated though various mechanisms have been proposed.^{24-28,32-34} Although

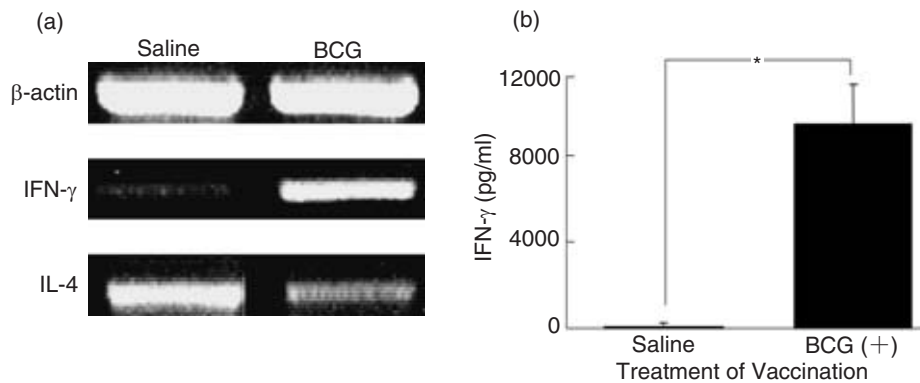


Fig. 7 The effect of BCG vaccine on splenic CD4⁺ T cells. **(a)** RT-PCR analysis of splenic CD4⁺ T cells obtained from saline- or BCG-injected mice. **(b)** Levels of IFN-γ measured by ELISA in the culture supernatants of splenic CD4⁺ T cells from saline- or BCG-inoculated mice. Data are the means $\pm$ SEM from four mice in each group (* $p < 0.01$).

the BCG vaccine is used clinically, few studies have demonstrated that subcutaneous inoculation with BCG suppresses allergic inflammation of the airway in OVA-sensitized mice. The potential to inhibit inflammatory responses was much lower in subcutaneously immunized mice than in mice that were immunized via the intranasal, intravenous or intraperitoneal route.^{25,35}

We selected 1×10^5 CFU as the dosage of BCG for this study, based on the difference in weight between mice (6 week) and neonatal human infants, who are vaccinated subcutaneously with 10^7 – 10^8 CFU of BCG.^{21,36} Moreover, even a low dose of BCG vaccine caused a Th-1 dominant response in spite of the fact that BALB/c mice have a genetic Th2 bias. Acosta *et al.* conducted a histopathologic study of BALB/c mice inoculated with BCG (1×10^6 CFU) by different routes, oral, intravenous and subcutaneous.³⁷ When the subcutaneous route was used, granuloma lesions developed only at the site of inoculation, whereas there was systemic dissemination when the oral or intravenous route, especially the latter, was employed. Realistically, the administration of BCG orally and intravenously, including the intraperitoneal and intranasal routes, is not practical because of the risk of infection. Our results might contribute directly to immunotherapy. In this study, we demonstrated that vaccination with BCG in OVA-sensitized mice significantly inhibited allergic inflammation of the airway and the effect was caused by BCG-sensitized CD4⁺ T cells. These cells produced large amounts of IFN-γ, and might change cytokine profiles from a Th2 to Th1-type immunity. Some reports demonstrated that IFN-γ gene transfer inhibited allergic inflammation,^{38,39} and that this effect was dependent on the IL-12 and STAT4 signaling pathways.⁴⁰ RT-PCR analysis demonstrated a high-level of gene expression of IFN-γ in CD4⁺ T cells from BCG-vaccinated mice. However,

there was no significant difference in IL-4 levels in sera between the PBS and OVA exposed group. These findings indicate that exposure to OVA might change the Th1/Th2 balance in the lung, but not in the systemic circulation. Zuany-Amorim *et al.* suggested that regulatory T cells generated by mycobacteria treatment had the potential to suppress inflammation of the airway through the subcutaneous injection of Mycobacterial Ags, *M. vaccae*, and did not alter IL-4 levels in BAL fluid.⁴¹ In a recent study, adoptive transfer of Th-1-like regulatory T cells markedly reduced the development of allergic inflammation.⁴² The involvement of a mechanism other than simple changes in the balance between Th1 and Th2 was suggested.

Chemokines, a group of specialized cytokines, play essential roles in the recruitment of leukocytes to sites of inflammation.⁴³ For example, Eotaxin which is thought to play an important role in the recruitment of eosinophils to airways and TARC, a selective chemoattractant of Th2 cells, is a reasonable candidate for a key regulator of Th2-mediated inflammation in allergic asthma.^{44–46} Our data revealed that gene expression of TARC as well as Eotaxin in the lung was down-regulated by the vaccination. However, the mechanism by which the BCG vaccine inhibited these chemokines is still unknown. Accordingly, further investigations on regulatory T cells and chemokines are needed.

In conclusion, we demonstrated that the subcutaneous inoculation of low dose BCG vaccine attenuated allergic inflammation of the airway in BALB/c mice. The expression of IL-4 as well as Eotaxin and TARC was down-regulated in the vaccinated mice, while that of IL-12 and IFN-γ was up-regulated. Moreover, the transfer of splenic CD4⁺ T cells, which produced a large amount of IFN-γ, prevented the development of allergic inflammation. The BCG vaccine

could potentially be used for immunomodulation in the treatment and prevention of bronchial asthma.

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