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Effects of BCG on ovalbumin-induced bronchial hyperreactivity in a guinea pig asthma model

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To test the effects of Bacillus Calmette-Guérin (BCG) on ovalbumin (OVA)-induced airway hyper-reactivity in guinea pigs, a total of 40 young guinea pigs was individually vaccinated subcutaneously with 0.2 mL of 2% OVA, 50 μg BCG, or a mixture of OVA and BCG (OVA+BCG). Airways were sensitized using nebulization with 1% OVA for 3 min once a week for two applications, followed by 2% OVA nebulized challenge for 3 min 1 week after the last application. Different concentrations of methacholine were used to detect airway hyperreactivities. At the third week, the guinea pigs were nebulized with either methacholine or OVA to test airway hyperreactivity. The OVA-vaccinated group presented with severe airway hyperresponsiveness after OVA and methacholine challenges; the BCG-vaccinated group showed mild airway hyperreactivity; and the OVA+BCG group showed the least amount of airway hyperreactivity. Lung histopathology in all groups, except the OVA+BCG-vaccinated group, showed severe thickening of the alveolar walls which became firmly fibrotic, and narrowing of the alveolar spaces was also noted. The guinea pigs in the OVA+BCG-vaccinated group had similar pulmonary morphology with that of naive guinea pigs, and had mild cell infiltration in the alveolar wall. The results of the skin biopsies at 6 h (2% OVA, 0.05 mL) and 36 h (20 μg PPD, 0.05 mL) after purified protein derivative (PPD) inoculation showed that infiltration of eosinophils and activation of $CD4^+$ T-cells occurred in the OVA-vaccinated group. In the BCG-vaccinated groups, infiltration of $CD4^+$ T-cells, $CD8^+$ T-cells and macrophages occurred. OVA-specific IgG2 increased in the BCG-vaccinated groups after OVA-induced airway hyperreactivity occurred. The peripheral cell subpopulation showed that there was obviously increased activation of $CD4^+$ and $CD8^+$ T-cells in the OVA+BCG-vaccinated group. The phagocytic activity of macrophages also increased in both BCG- and OVA+BCG-vaccinated groups. The prevention of OVA-induced airway hyperreactivities using BCG vaccination in conjugation with OVA in these young guinea pigs indicated that it might be a good approach to avoid allergic reactions in humans.

Key words: BCG, bronchial hyper-reactivity, guinea pig, ovalbumin

Inhalation of antigen ovalbumin (OVA) in the absence of adjuvants has been demonstrated to induce airway hyperresponsiveness and obstruction in OVA-sensitized guinea pigs [1,2]. This airway response reveals the release of preformed and newly generated mediators from activated mast cells in bronchial mucosa. This hypersensitive response immediately reached the maximum 15 to 30 min after challenge, and lasted for as long as 2 h after the antigen challenge [3]. This late-phase response recurred with diminished intensity 2 to 3 days after the challenge [4]. The late-phase reactions were accompanied by an increased responsiveness of the airways to a variety of stimuli such as inhaled methacholine and histamine [5,6].

Results of recent histopathologic studies of asthmatic lungs suggested that there was a correlation between progressively mixed inflammatory leukocyte infiltration and late-phase asthmatic reactions [7,8]. Experimental animal models of asthma showed a significant increase in the number of eosinophils in lung tissue and bronchial lavage fluid [9-11].

Bacillus Calmette-Guérin (BCG) has an antigenic component that can stimulate the helper T lymphocyte type 1 (TH_1) response. Helper T lymphocytes in the blast cell phase treated with a bacterial antigen like BCG selected the TH_1-like growth route. However, treatment of lymphoblasts with an allergenic peptide antigen like OVA induced a helper T lymphocyte type 2 (TH_2)-like growth route [12]. One researcher found that TH_1 cells

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released cytokines such as interleukin-2 (IL-2) and interferon-γ (IFN-γ) to promote the phagocytic ability of macrophages and nature killer cells (NKs), and TH_2 cells released IL-4 and IL-5 to stimulate the maturation of mast cells and eosinophils, which were embedded vesicles with allergic mediators [13]. In our study, the combination of BCG with OVA injected subcutaneously was used to evaluate the effects on OVA-induced airway inflammation and hyperreactivity. The reactions to OVA-specific IgG1 in guinea pigs were also investigated. Studies of allergic diseases in animal models play pivotal roles for TH_2 cytokine-producing cells in the development of allergic responses. Strategies that are more recent have been aimed at switching allergen-specific TH_2 responses to TH_1, because TH_1 and TH_2 cytokines are thought to be mutually antagonistic [14,15]. The purpose of this study was to examine whether BCG inoculation with specific allergen down-regulated allergen-specific TH_2-like immune responses.

Materials and Methods

Forty 150 g male Dunkin-Hartley guinea pigs were bought from the National Laboratory Animal Breeding and Research Center in Taiwan. The animals were raised to 450 g in an animal experimental room of Cathay General Hospital, Taipei. Pyrilamine, OVA, and methacholine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The ketamine was obtained from Parke-Davis Inc. (Elk Grove Village, Ill, USA).

Pulmonary function evaluated in guinea pigs

The OVA group was injected subcutaneously with 0.2 mL of a 20-mg/mL OVA solution when they were 150 g. The BCG group was injected with 50 μg of commercial BCG vaccine subcutaneously when they were 150 g. The OVA+BCG group was injected with volumes of both the above-described OVA and BCG when they were 150 g, while the control group was not treated with anything until after they reached 450 g. When the guinea pigs reached 450 g, each group of 10 guinea pigs was sensitized by exposure to aerosolized OVA (1% w/v, in isotonic sodium chloride solution) with a facial mask for 3 min on two occasions separated by 7 days. After the guinea pigs were sensitized, bronchial hyper-responsiveness was induced. The time interval from BCG vaccination to first OVA nebulized challenge was approximately 2 weeks.

Seven days after the second induction, the four groups of guinea pigs were challenged with 2% OVA-saline solution for 3 min and covered with facial masks. In order to prevent anaphylactic shock, 10 mg/kg of pyrilamine was injected intraperitoneally 30 min before OVA challenge, then the immediate bronchial responses were measured for 10 min, as were the late-phase bronchial responses at 6 h and 24 h, respectively [16].

Airway resistance was measured using an infant endoesophageal tube filled with isotonic sodium chloride solution to evaluate changes of pressure in the trachea.

The airway responses to OVA were determined in conscious, nose-breathing guinea pigs by measuring pulmonary resistance, dynamic lung compliance, and tidal volume. After intramuscular administration of ketamine (30 mg/kg), the guinea pigs were placed in the supine position. The pharynx and epiglottis were swabbed with 1% lidocaine to prevent gagging, and a water-filled feeding tube (Terumo, Tokyo, Japan) was placed in the esophagus to measure the intrathoracic pressure. Lung volume was measured using the Lab Pulmonary Evaluation and Diagnostic System (Model PC6386 WGS, AT&T, Medical Associated Services, Hatfield, PA, USA). The concentrations of methacholine used for airway provocation were 0.075, 0.15, 0.3, 0.625, and 1.25 mg/mL in isotonic sodium chloride solution, respectively. Methacholine was prepared in isotonic sodium chloride solution and delivered through a Mefar MB3 dosimeter.

Prior to methacholine challenge, all animals received five inhalations of isotonic sodium chloride solution. Pulmonary function tests were performed before and after inhalation of isotonic sodium chloride solution, and the results were taken as the baseline control value. If airway responses to isotonic sodium chloride solution did not differ from the baseline, the animals would be accepted for methacholine challenge. For the methacholine challenge, five breaths of serially diluted methacholine were given to each guinea pig, with pulmonary function measurements taken 3 min after each challenge. Both tidal volume and airway resistance were measured for 15 min after each challenge.

Determination of the effects of BCG immunization on anti-OVA IgG antibody titers

Blood samples were obtained by heart puncture at the beginning and end of the experiment. Serum anti-OVA IgG antibodies titers were determined using an enzyme-linked immunosorbent assay (ELISA). Microtiter plates (Nunc Laboratories, IL, USA) containing 100 μL OVA (grade 5, Sigma, Germany) at a concentration of 0.1 mg/mL, which were sealed with parafilm, were stored at 4 °C overnight. The plates were washed three times with a saline-Tween 20 solution before adding a

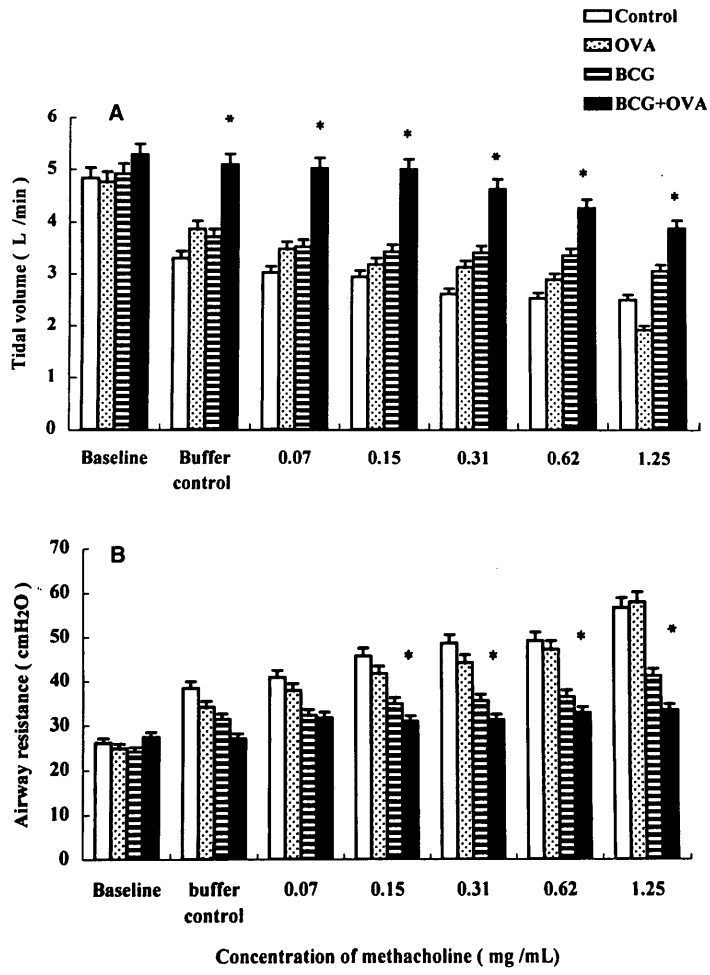

Fig. 1. Airway hyper-reactivity to different concentrations of methacholine aerosol administration among four groups of guinea pigs. **A.**, Tidal volume. **B.**, Airway resistance. Data are means ±SEM of 10 animals. $^{*}p \leq 0.05$, as compared with the control group.

1% low-fat milk powder/phosphate buffered saline (PBS) solution for blocking. This reaction proceeded at room temperature for 2 h. After the addition of a 1:9 dilution of guinea pig serum, the plates were incubated for 6 h at 4 °C. The plates were washed three times before the antibody (horse-radish peroxidase-conjugated goat anti-guinea pig IgG, 1:3000 [KPL, MO, USA]) was added. Following a 1-h incubation at 37 °C and three washes with 0.05% PBS-Tween 20 (PBS-T), the enzyme substrate, phenylenediamine (OPD 0.4 mg/mL; containing 0.03% hydrogen peroxide), was added. The reaction was stopped with 50 µL 4 N H_2SO_4 after 15 min, and the optical density was measured at 492 nm in a multiscan photometer (model A-5682, SLT Lab Instruments, Salzburg, Austria). The groups which received immunization using OVA combined with aluminum hydroxide (OVA/Alum) and OVA combined with completed Freud's adjuvant (OVA/CFA) were taken as positive control subjects, and the naive as negative control subjects.

Histopathological examination with immunocytochemical staining

The presensitized immune responses were induced in young guinea pigs using subcutaneous injections of OVA and BCG. For allergic skin reactions, 0.1 mL of a 2% OVA solution was used with 20 µg purified protein derivative (PPD) being given to each guinea pig. After an additional subcutaneous injection with a small volume of OVA, samples for the late-phase allergic reactions were taken using punch needle biopsy. The BCG-immunized guinea pigs showed delayed-type hypersensitivity after PPD was injected subcutaneously 24 h after BCG immunization.

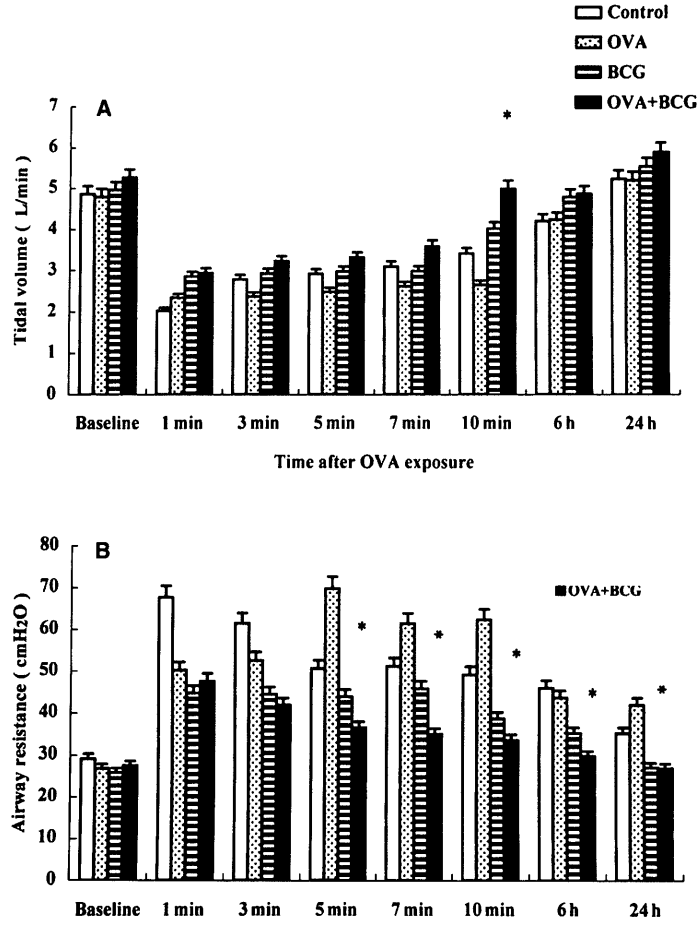

Fig. 2. Changes in pulmonary function immediately (early-phase) after and 6 h (late-phase) after primary sensitization by OVA using aerosol administration of antigen in the guinea pigs. **A.**, Tidal volume. **B.**, Airway resistance. Data are means ±SEM of 10 animals. *$p \leq 0.05$, as compared with the control group.

The punched skin samples were embedded in frozen-section gel, then fast frozen in liquid nitrogen. Thin-cut samples on a slide were fixed with ethanol and acetone, and rinsed using PBS. Then the blocking reagent A, B (Zymed®, CA, USA) was added to fill the non-antigenic region for 40 min. The sample was washed with PBS; CD3, CD4, CD8, or anti-major histocompatibility complex (MHC), while class I and II monoclonal antibodies (Serotec, Oxford, England) were placed individually in each tissue sections. The reactions were allowed to progress at 4 °C overnight. After washing the covering antibodies, the biotinylated second antibody (Zymed®) was added and washed off 10 min after it was added. Then streptavidin peroxidase was added and allowed to react for 10 min. The reagent was then washed off and chromogen 3,3'-Diaminobenzidine tetrahydrochloride (DAB) (Zymed®) was used to express the target CD marker under a microscope. Hematoxylin was used as the counter-stain dye to differentiate the position of the nucleus from the CD markers on the cell membrane.

Immunofluorescent staining and flow cytometry analysis

The details of immunofluorescent staining and FACScan analysis were described by Hayes *et al* [21]. Briefly, peripheral blood mononuclear cells (PBMCs) were incubated with anti-CD3, CD4, CD8 monoclonal antibodies (MoAb), conjugated with fluorescein isothiocyanate (FITC) 15 min for direct staining. After a 15-min incubation, cells were washed and incubated with CD11b/18 (Mac-1)(Boehringer, Germany), the MHC-class I and II MoAb (Serotec, Oxford, UK) for 1 h at 4 °C. Then the cells were washed and incubated with goat anti-mouse IgG1 MoAb (Zymed Lab, CA, USA), as a secondary antibody for indirect staining,

for 30 min at 4 °C. Dichlorofluroreceín diacetate (DCFH-DA) (Sigma, Germany) was used as a phagocytic indicator, when cells ingested it and the peroxidase inside the phagosome hydrolyzed DCFH-DA into DCF and DA. DCF emitted a FITC-like fluorescence. The cells were analyzed by flow cytometer using a FACScan system (Beckton Dickinson Worldwide, San Jose, CA, USA) operating at a laser wavelength of 488 nm and power output of 15 mW. For each experimental condition, 5000 cells were analyzed.

Statistical analysis

The results were analyzed using paired Student's *t*-test to compare pulmonary function between the same groups before and after immunization and with unpaired Student's *t*-test to compare the pulmonary function between different groups of guinea pigs. Data are expressed as the mean ±standard error of the mean (SEM), compared with the data from the control group.

Results

Immunization with BCG/OVA mixture reveals protective effects in pulmonary function in an OVA-sensitized guinea pig asthma model

After 5 min of methacholine inhaled challenge, bronchial resistance in the OVA and control groups increased. Using 1.25 mg/mL of methacholine, the bronchial resistance in the OVA group was 50% higher than that in the OVA+BCG group. Even the resistance in the BCG group was 20% higher than that in the OVA+BCG group (Fig. 1A,B).

When guinea pigs were exposed to aerosolized OVA three times (once a week for 3 weeks), 93% (37/40) of the animals became sensitized to OVA. A time-course study showed that there were dual-phase pulmonary responses in OVA-sensitized guinea pigs upon re-exposure to OVA. The tidal volume and pulmonary resistance changed immediately (early phase) and at 6 h (late phase) after challenge. The mean tidal volume in the OVA+BCG group was 20% higher than those of the other three groups. Airway hyperreactivity in the OVA group showed the lowest tidal volume and bronchial resistance with a methacholine concentration of 1.25 mg/mL.

Provocation with OVA showed that there was a significant protective effect after OVA+BCG immunization. During the first few min after nebulized OVA challenges in the time course study, the tidal volume of the OVA+BCG and BCG groups were 24% higher than those of the other two groups. At 10 min, volume in the BCG+OVA group was two-fold higher than that in the OVA group. The bronchial resistance of the OVA group increased 30 cm to 50 cm H_2O at 1 min after OVA provocation, when compared with baseline values. The bronchial resistance of the control group showed a 30-cm to 70-cm H_2O at 1 min after OVA challenge, but recovered quickly thereafter. Airway hyper-reactivities in the BCG and OVA+BCG groups appeared to be much better than those in the other two groups (Fig. 2A,B).

Histopathological changes after OVA sensitization and the dermatological immune response in immunochemical staining

After nebulization with OVA, the alveolar cells thickened, especially in the OVA and control groups. The structure of the alveoli was damaged, including an accumulation of inflammatory cells and secretory fibrin, and loss of microvilli on the bronchi. The eosinophils were chemoattracted to the space between bronchial smooth muscle and lamina propria (Fig. 3A). The group of guinea pigs immunized with BCG also had obstruction of the alveoli. Inflammatory cells accumulated as nodules, surrounded the bronchi, and caused progressive emphysema. The OVA+BCG group showed little hyperplasia on the bronchial epithelia, with no loss of alveolar structure (Fig. 3B). After the histoimmunochemical staining of CD4, CD8 on the surface of lymphocytes, great expressions on the lung tissues of the BCG-immunized groups were noted (Fig. 3C, D). There were more $CD4^+$, $CD8^+$ lymphocytes in the OVA+BCG group than in the BCG only group (Table 1).

Cell subpopulations in lung tissues and skin biopsies were counted. Severe eosinophilic infiltration was preceded in the OVA-immunized groups, in both OVA and OVA+BCG group; large amounts of macrophages and lymphocytes appeared inside the skin indurations in two BCG-immunized groups after PPD induction (Table 1).

Changes in OVA-specific IgG antibodies before and after OVA sensitization

OVA-specific IgG, IgG1, and IgG2 were studied before and after OVA sensitization. The OVA-specific antibodies were elicited 4 weeks after OVA exposure of the guinea pigs in all groups. The specific IgGs increased in the BCG group as compared to the other three groups (Fig. 4A).

OVA-specific IgG1 increased greatly in the BCG group. However, increases in both the control and OVA groups were mild, and trivial in the OVA+BCG group

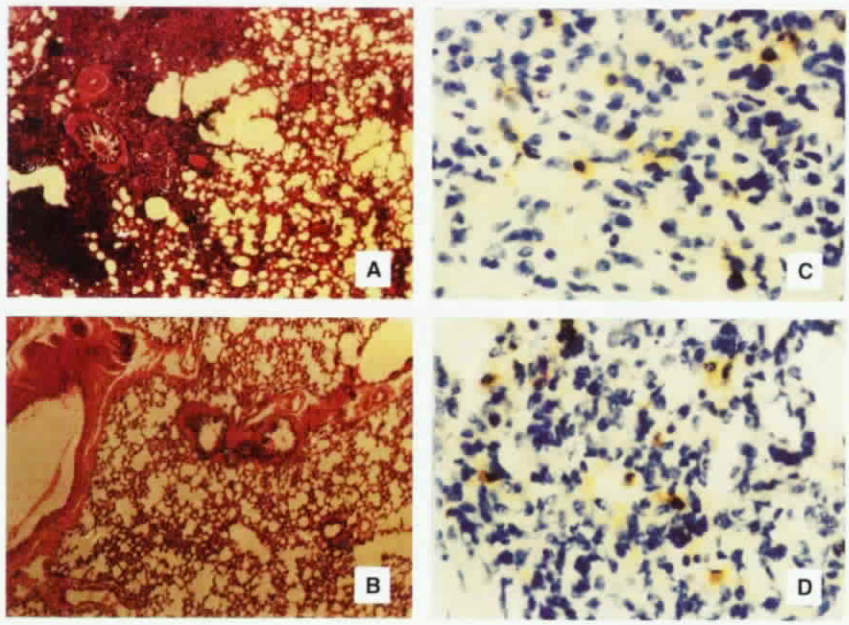

Fig. 3. Histopathological changes of lung tissues and histo-immunochemical staining of inflammatory cells on lung tissues. **A.,** Lung tissue of OVA-immunized group (HE stain, 100x). **B.,** Lung tissue of the OVA+BCG group (HE stain, 100x). **C.,** Infiltration of $CD8^+$ lymphocytes in lung tissue of BCG-immunized groups (400x). **D.,** Infiltration of $CD4^+$ lymphocytes in lung tissue of BCG-immunized groups (400x).

Table 1. Cell subpopulations in lung tissue and skin biopsies

	Neutrophil[a]	Eosinophil[a]	Macrophage[a]	CD4[b]	CD8[b]
OVA skin reaction (6 h)					
Control	+	+	-	16 (±1.2)	7 (±0.6)
OVA	+++	+++	-	18 (±2.6)	5 (±0.9)
BCG	+	++	-	21 (±1.7)	13 (±2.1)
OVA+BCG	++	++	+	27 (±2.4)	15 (±2.7)
PPD skin reaction (48 h)					
Control	+	-	+	18 (±3.0)	9 (±2.5)
OVA	+	-	+	23 (±2.1)	11 (±1.9)
BCG	++	-	+++	67 (±4.5)	39 (±4.7)
OVA+BCG	++	-	+++	85 (±8.2)	61 (±5.3)
OVA lung challenge (6 h)					
Control	+	+++	+	42 (±2.2)	21 (±2.9)
OVA	+	+++	+	43 (±3.8)	25 (±3.2)
BCG	++	++	++	45 (±5.3)	38 (±4.7)
OVA+BCG	++	+	++	50 (±5.6)	44 (±6.1)

Abbreviations: OVA = ovalbumin; BCG = Bacillus Calmette-Guerin

[a]+ indicates 0-20 cell numbers, the mean value of counting 200 leukocytes in six high power fields (HPFs); ++ indicates 21-50 cell numbers; +++ indicates more than 50 cell numbers.

[b]Data are means ±SEM, cell numbers counted in six HPFs, 200 leukocytes per HPFs.

(Fig. 4B). In the two BCG-immunized groups, OVA-specific IgG2 increased more than that of the OVA group. When the IgG2/IgG1 ratio was compared among the four groups after OVA challenge, the value of the control, OVA, BCG and OVA+BCG groups were 1, 1, 3.26 and 6.15, respectively. The BCG-vaccinated groups showed the most obvious elevation of IgG2 among the groups. OVA+BCG groups were the highest (Fig. 4C).

Cell subpopulations in peripheral blood

A FACScan analyzer analyzed cell subpopulations in

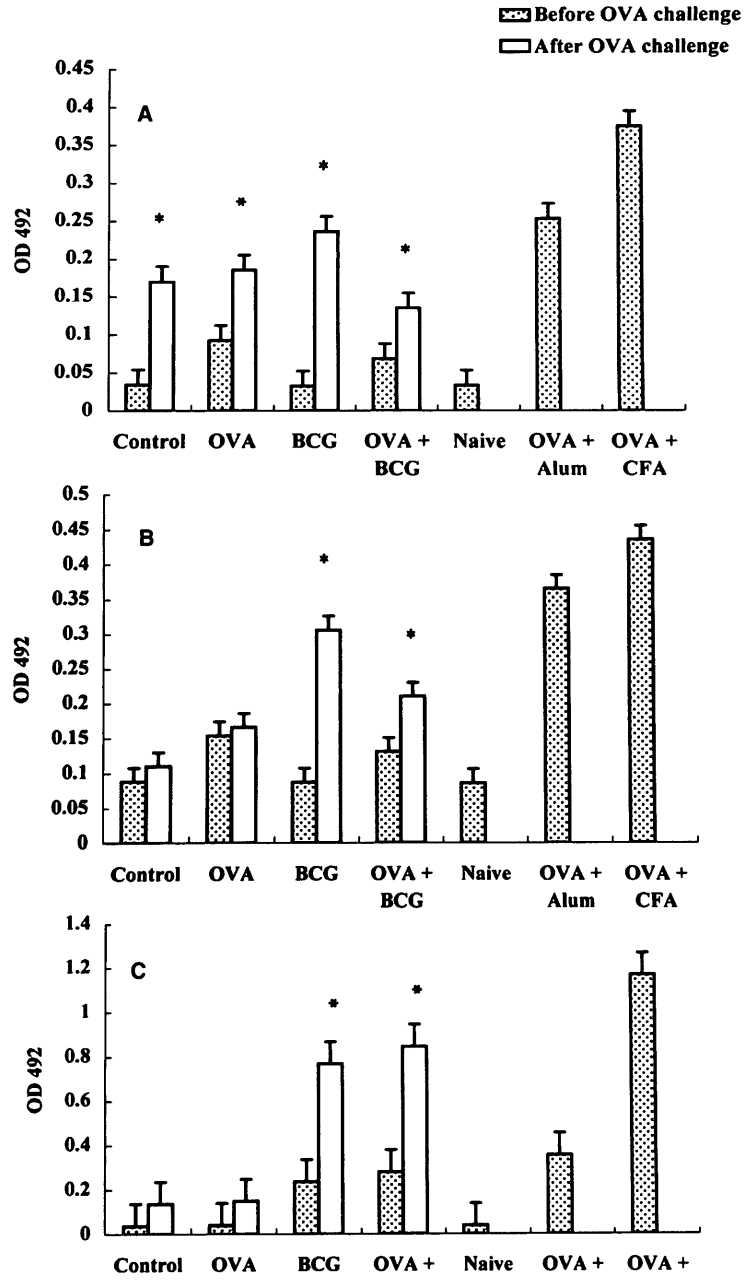

Fig. 4. Changes of OVA-specific antibody before and after aerosol administration of OVA. The guinea pigs were immunized with OVA, BCG, OVA+BCG, and isotonic sodium chloride solution for 4 weeks, and the sera were collected before and after the nebulization of OVA. **A.**, Serum anti-OVA IgG. **B.**, Anti-OVA-specific IgG1. **C.**, Anti-OVA-specific IgG2. Data are means ±SEM of 10 animals. $^*p \leq 0.05$, as compared with the control group. Alum = aluminum hydroxide; CFA = complete Freud's adjuvant.

PBMCs. More than 90% of $CD3^+$ cells were found in the PBMCs. The results showed that 50% and 70% of cells expressed were MHC class II in $CD4^+$ and MHC class I in $CD8^+$ lymphocytes, respectively, for the OVA+BCG group. The values were significantly higher than those for the other groups at p value lesser than 0.05.

Phagocytic activity was analyzed using dual staining with the CD11b/18 surface markers and DCFH-DA. The results showed that phagocytic activity was

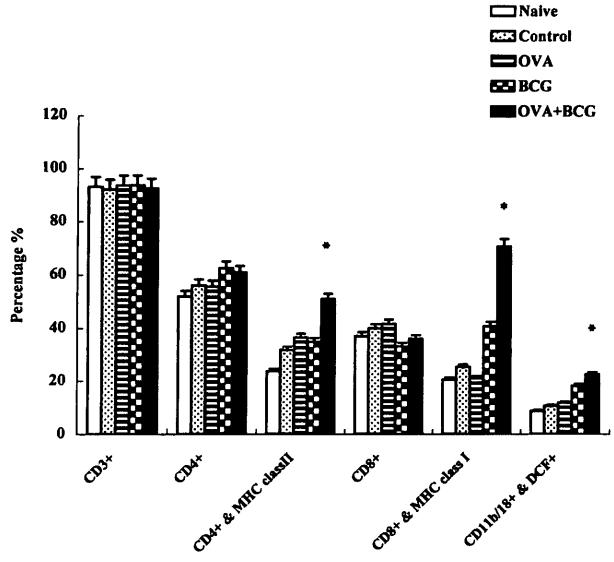

Fig. 5. Cell subpopulations among four groups of guinea pigs after OVA nebulization. Data are means ±SEM of 10 animals. $^{*}p \leq 0.05$, as compared with the control group.

great in the OVA+BCG group, being significantly higher than those of the control and OVA groups (Fig. 5).

Discussion

We developed an animal model of bronchial asthma using guinea pigs, in which both early- and late-phase responses were successfully achieved by aerosol administration of OVA to airways of conscious guinea pigs. Subcutaneous injections with OVA or BCG alone, or OVA combined with BCG in young guinea pigs subsequently challenged with nebulized OVA-saline solution produced significantly protective effects from bronchial spasm after BCG immunization. Using BCG alone produced less protective effects than did BCG combined with the allergen, OVA. This is probably because the allergen combined with BCG gave antigen-presenting cells and lymphocytes more specific signals for the transductional pathway [12]. Effectively suppressing the elicitation of airway resistance and maintenance of tidal volume from allergen-caused airway hyperreactivity was especially well demonstrated in the OVA+BCG group.

Airway resistance in the control and OVA groups differed. The control group reached the highest point faster than did the OVA group. The hypothesis was that when guinea pigs were injected with OVA subcutaneously, anti-OVA IgG antibodies were generated. The control group did not become sensitized previously with OVA and no anti-OVA antibodies were generated, so bronchial hyper-reactivity occurred more quickly than in the OVA group according to the time course of OVA challenge [14].

The protective effects of BCG on OVA-induced airway spasm obviously occurred in the late-phase. Airway resistances at 6 h and 24 h after OVA challenges had decreased to normal levels in both the BCG and OVA+BCG groups. It has been reported that the late-phase reactions were closely related to the activation of lymphocytes and eosinophils between 4 h and 8 h after allergen induction [17], and many inflammatory mediators and cytokines were released during this period. The BCG-immunized groups showed no significant changes of pulmonary function during the late-phase reaction when compare with the naive group. The results probably occurred because BCG-mediated TH_1-like responses effectively suppressed the release of IL-4, IL-5, and leukotrienes [18].

The pathology of lung tissue showed that the alveolar wall lost the monolayer cell structure and filled with inflammatory cells, such as neutrophils and eosinophils, after OVA sensitization [19]. The lymphocytes gathered as masses near the bronchial area in the BCG group. Because of the destruction of the alveolar structure, the air-exchange areas became fibrous and consolidated with compensatory emphysema. The OVA+BCG group showed less destruction of the lung tissue; very mild inflammation responses and leukocytes rarely accumulated, and alveolar wall remained almost like the original structure. On the other hand, guinea pigs in the BCG alone group showed less protective effects to allergen induced bronchial inflammation. Many inflammatory cells, such as macrophage and lymphocytes, were induced to infiltrate inside the alveolar space (figure not shown). These pathological changes showed the consolidation of vital air exchange space in the control, OVA and BCG alone groups. The OVA+BCG group provided nice protection in allergen-induced airway inflammation. Changes of lung structures caused by the allergen and previous immunization were compatible with the alteration of vital capacity. Eosinophilias in the airway inflammatory area were inhibited by BCG immunization, especially in the OVA+BCG group [20,21].

$CD4^+$ T-cells were chemoattracted to inflamed areas of the bronchi; fewer $CD8^+$ T-cells existed in the bronchial area without BCG immunization. The accumulation of $CD4^+$ and $CD8^+$ lymphocytes and phagocytes provided good resources of cytokines as chemotactic factors. These are compatible with results of another researcher who reported that their migration from peripheral blood to inflamed areas depends on the release of chemoattractant factors from phagocytes and inflammatory cells [22].

According to previous reports, OVA-specific IgG1 increased after allergen stimulation. In our study, OVA-specific IgG1 increased after OVA nebulization in all guinea pigs, especially in the control and BCG groups; but the IgG1 [23,24] level had no correlation with allergic symptoms in the experimental animals. This phenomenon remains to be further clarified.

OVA-specific IgG2 increased more in the BCG-immunized guinea pig groups than in groups without BCG immunization. Similarly, in guinea pigs with OVA combined with aluminum hydroxide or OVA combined with CFA, the hyperimmune reaction caused an increase of OVA-specific IgG2. This increase in IgG2 production was probably due to B-cells being activated by BCG and CFA [12].

The negative control standard in the flow cytometry analysis was set by mouse IgG; the mouse antibodies did not react with the surface markers on the lymphocytes of the guinea pig. The BCG-immunized groups had greater expression of $CD4^+$ with MHC class II^+, and $CD8^+$ with MHC class I^+. After injecting allergens combined with BCG or BCG alone subcutaneously, activated lymphocytes migrated to allergen-induced inflammatory areas such as lungs, bronchi or trachea.

Our study showed that the utility of DCFH-DA in the BCG and OVA+BCG groups was higher than that in the other groups, which may be due to the phagocytic activity of macrophages and monocytes activated by BCG. The phagocytic ability was elevated specifically by OVA+BCG.

In summary, several mechanisms may be responsible for the suppression of allergen-induced airway hyper-reactivity in OVA-sensitized guinea pig immunized with BCG. This study showed several pieces of evidence that BCG immunization induced a PPD-like delayed immune response, although many undiscovered clues remain in the guinea pig animal model. The combination of the allergen with the immunogen, such as BCG, elevated the specificity of immune recognition of the antigen, better than when BCG was used alone [25]. Using BCG alone delayed hypersensitivity responses were induced, but the specificity to antigens may have been induced by the antigen cooperating with BCG. The availability of extensive safety data in humans suggests that BCG should be tested as a preventive agent for allergic diseases, and this method is currently undergoing clinical trials for this purpose.

Acknowledgment

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